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(54) **ISOPRENE SYNTHASE AND METHOD OF PREPARING ISOPRENE USING THEREOF**
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(30) **Foreign Application Priority Data**

Jul. 28, 2014 (KR) 10-2014-0095972

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C12N 9/88 (2006.01)
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C12N 15/74 (2006.01)
C12P 5/00 (2006.01)

(52) **U.S. Cl.**
CPC **C12N 9/88** (2013.01); **C12N 15/74** (2013.01); **C12P 5/007** (2013.01); **C12P 5/026** (2013.01); **C12Y 402/03027** (2013.01)

(58) **Field of Classification Search**
CPC . C12N 9/88; C12N 15/74; C12P 5/007; C12P 5/026; C12Y 402/03027
See application file for complete search history.

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(57) **ABSTRACT**

Provided are a novel isoprene synthase derived from sweet potato and a method of preparing isoprene using the same, and more specifically, a novel isoprene synthase derived from sweet potato, a gene encoding the isoprene synthase, a host cell transformed with the gene, and a method of preparing isoprene using the same. The isoprene synthase of the present invention may have higher isoprene productivity as compared to isoprene synthases known in the related art to thereby be effectively used in isoprene biosynthesis and preparation of an isoprene polymer using the same.

3 Claims, 21 Drawing Sheets
Specification includes a Sequence Listing.

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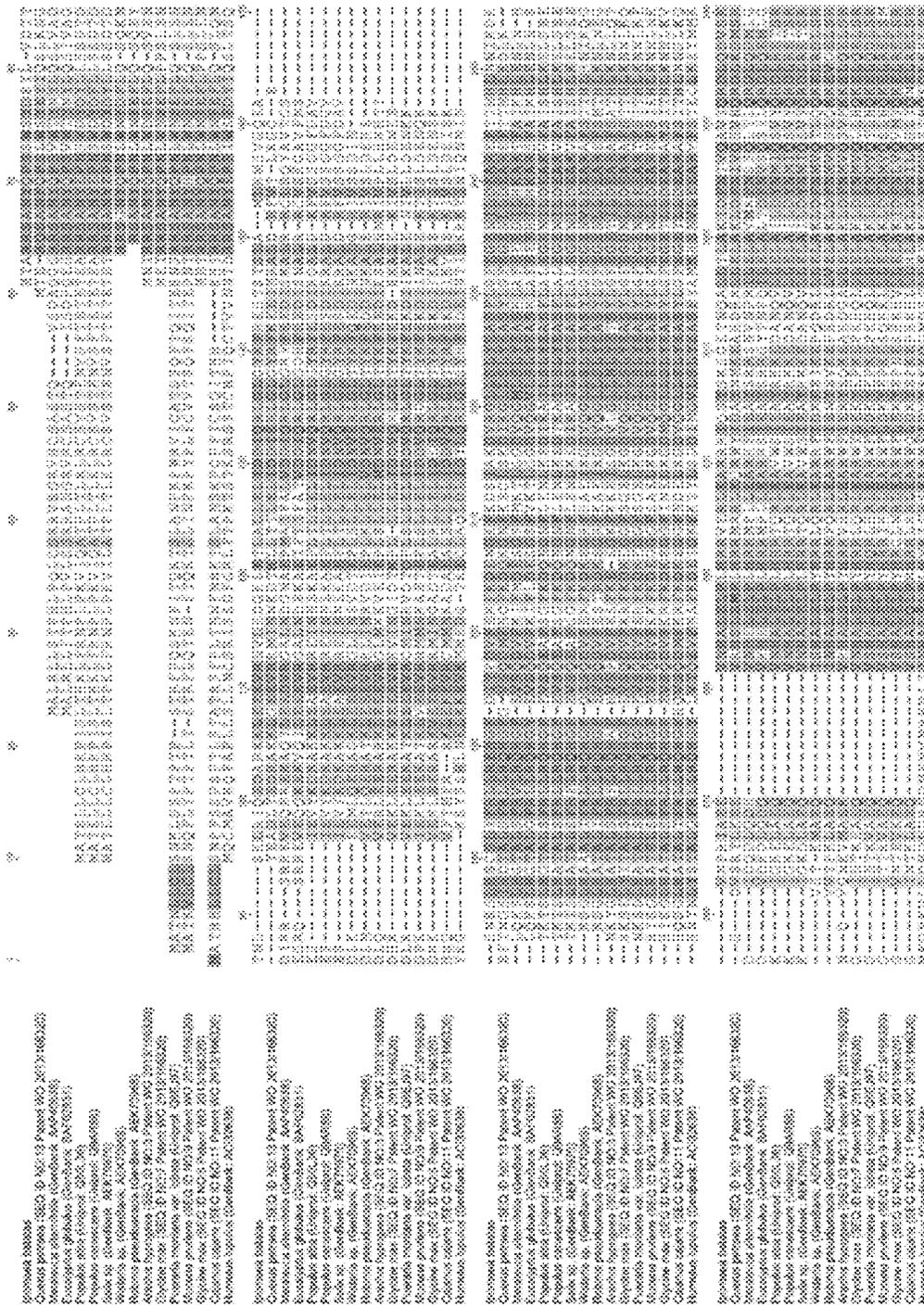
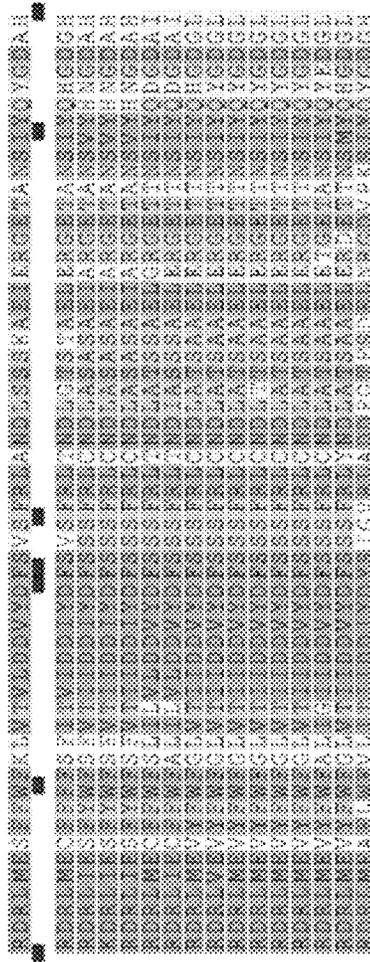


Fig. 1A



ipomoea batatas

- Quercus petraea (SEQ ID NO:13 Patent WO 2013/166320)
- Populus alba (Uniprot: Q50L36)
- Populus canescens (Uniprot: OMA066)
- Salix sp. (GenBank: AEK70870)
- Melaleuca alternifolia (GenBank: AAP40633)
- Eucalyptus globulus (GenBank: BAF02831)
- Vaccinium sp. (GenBank: AEK70969)
- Rubiana pseudocornuta (GenBank: AEK70968)
- Arachis hypogaea (SEQ ID NO:3 Patent WO 2013/166320)
- Glycyne max (SEQ ID NO:7 Patent WO 2013/166320)
- Pueraria montana var. lobata (Uniprot: Q6E387)
- Mucuna pruriens (SEQ ID NO:9, Patent WO 2013/166320)
- Glycyne max (SEQ ID NO:5 Patent WO 2013/166320)
- Cajanus cajan (SEQ ID NO:11 Patent WO 2013/166320)
- Humulus lupulus (GenBank: AC132038)

Fig. 2

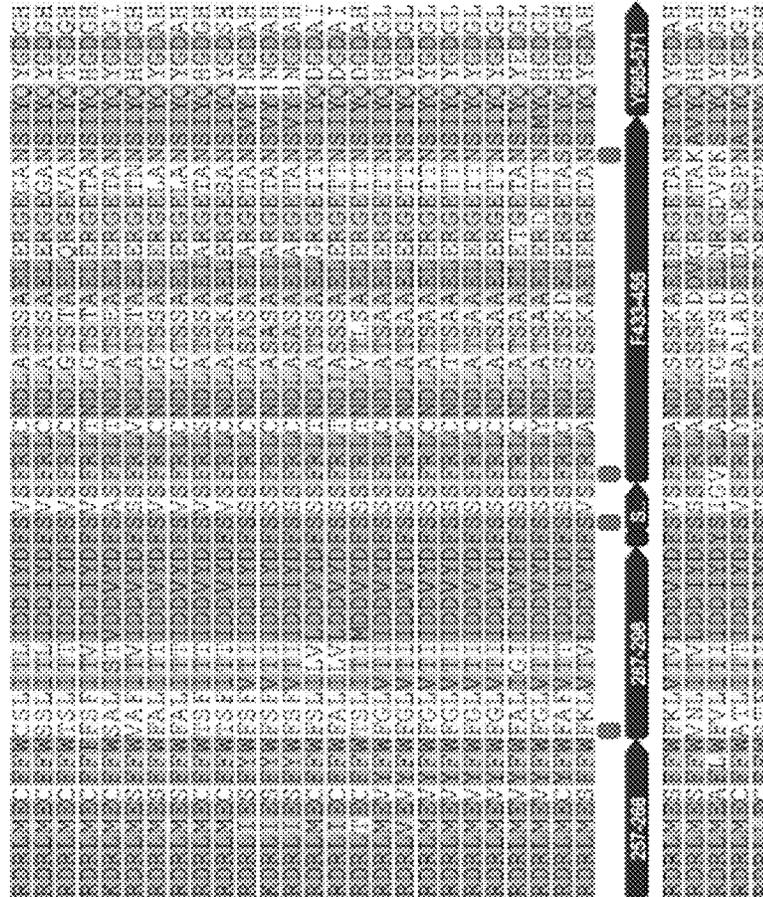


Fig. 3

1. *Tricyclene_Mexicago_truncatula_spl05LJB07ITPS4_MEO*
2. *Medicago_sativa_gf1555498871gblGAF701116086.1.0*
3. *Tricyclene_Lotus_japonicus_spl08727ITPS2_LOTJA_0*
4. S13. *Opuntia_1ps5*
5. *Fragaria vesca_gf470140811fneXP_004308033.1.0_NA*
6. *Morus_natalalis_gf981926327gblEXCZ3171.1.0_NA_8*
7. *Elaeocarpus*
8. *Elaeocarpus_guineensis_gf388282537gblFX134022*
9. *Populus_trichocarpa_vf89fHE95f89fHE96_POPTR_0_sc*
10. *Comene_Vitis_vinifera_vfA38153fA58155_VITV_0_sc*
11. *spl055LJgblSPS_POPAL.1*
12. *CACN56963NKGQ8AR86*
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14. *Sha_Mallemibia_1ps5_AAP40638.1*
15. *Sha_Egobius_1ps5_BAF03831.1*
16. *Eucalyptus_grandis_gf829086625gblKCV47670.1.0_*
17. *Sha_Wistaria_1ps5_AEK70969.1*
18. *Sha_Rquevotocacia_1ps5_AEK70988.1*
19. S3. *Ahyppogaea_1ps5*
20. S7. *Gmax_1ps5*
21. *spl08EJ9fJSPS_PUEML.1*
22. S9. *Mbructene_1ps5*
23. S5. *Gmax_1ps5*
24. S11. *Crajinis_1ps5*
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26. *Dalissis*
27. *Inermoea_babatas_gf245728787gblJP169673.1.0_NA*
28. *Sesamum_indicum_gf357335539gblJP845798.1.0_*
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30. *Comene_Maericaria_reculita_spl06RE8ITPS4_MATRE*
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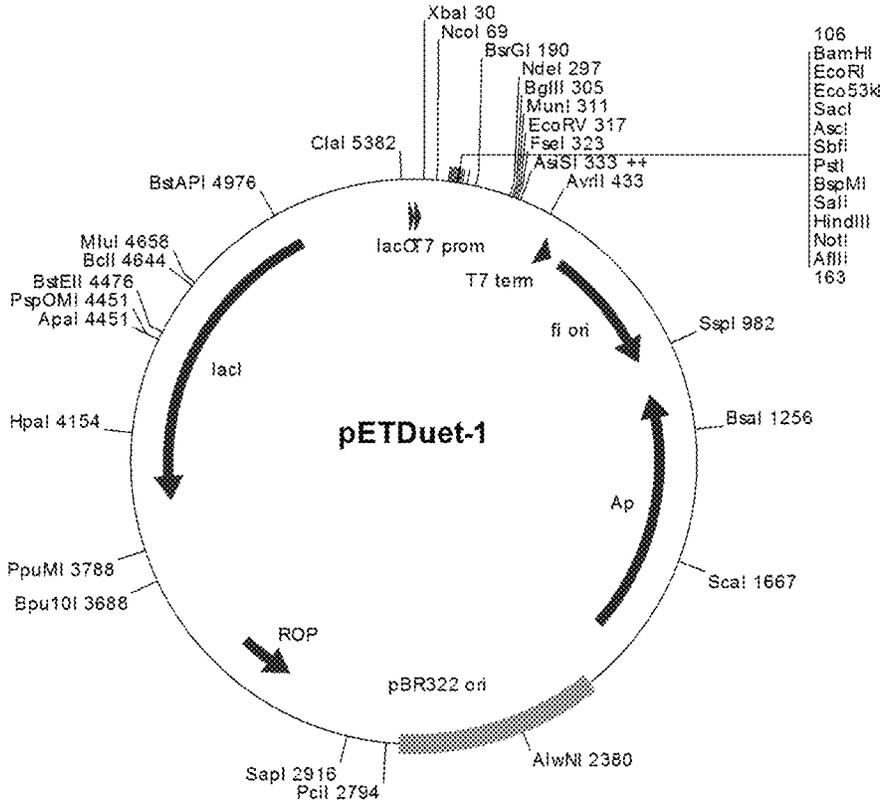


Fig. 6

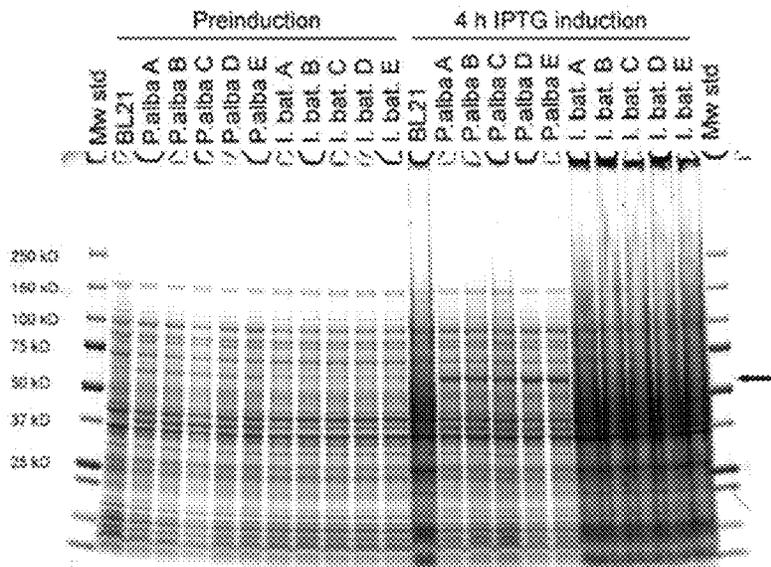


Fig. 7

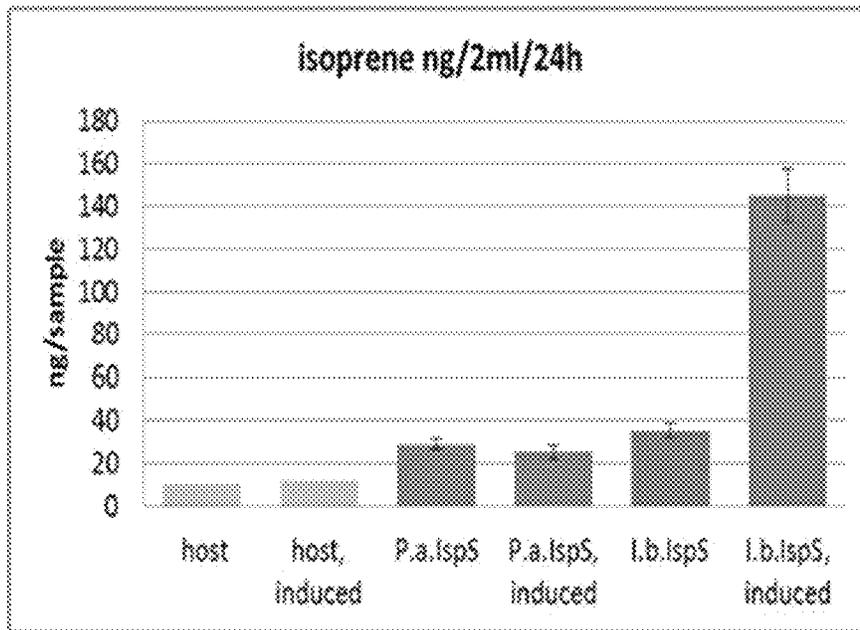


Fig. 8

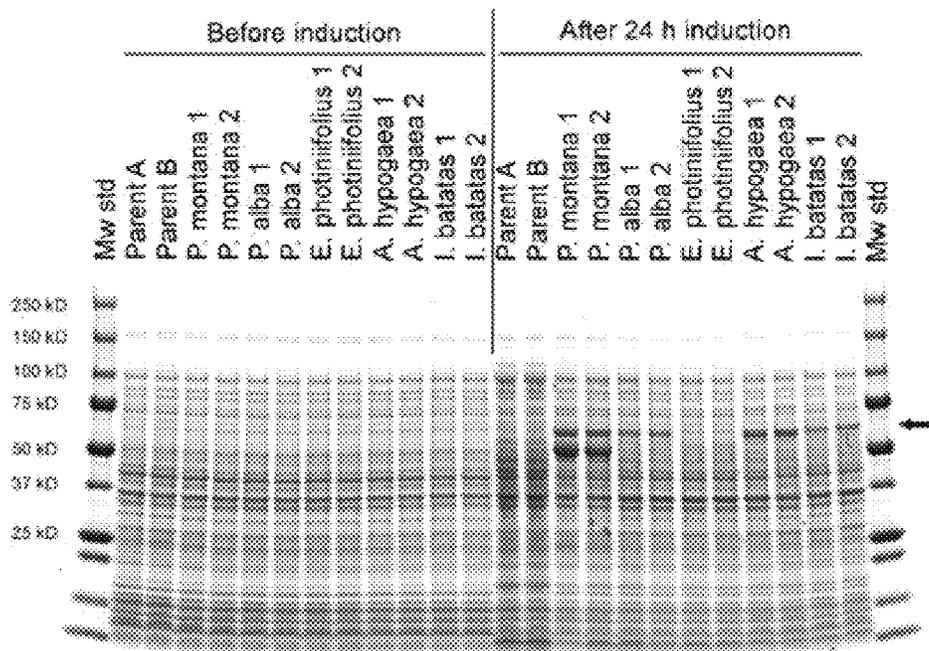


Fig. 9

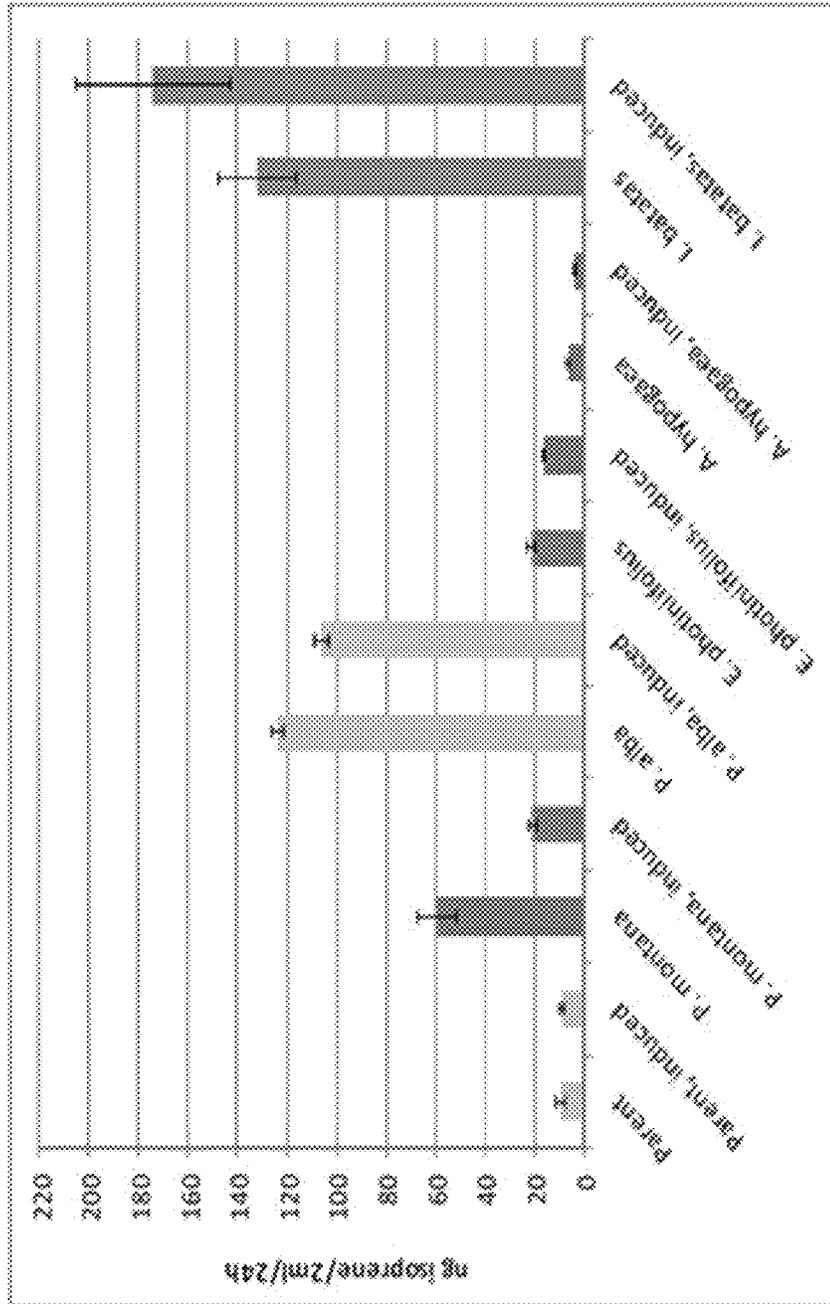


Fig. 10

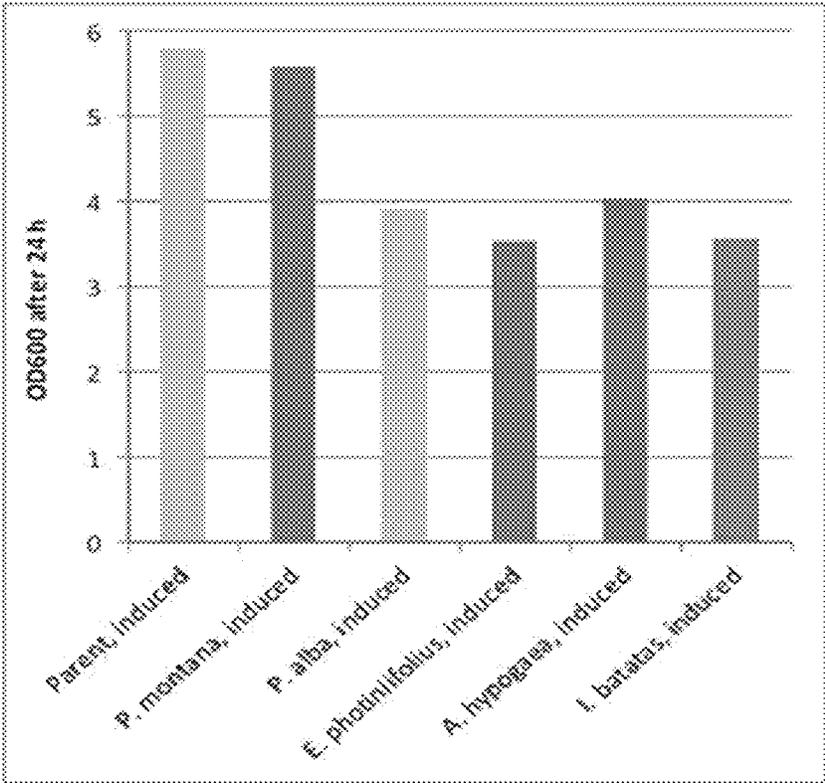


Fig. 11

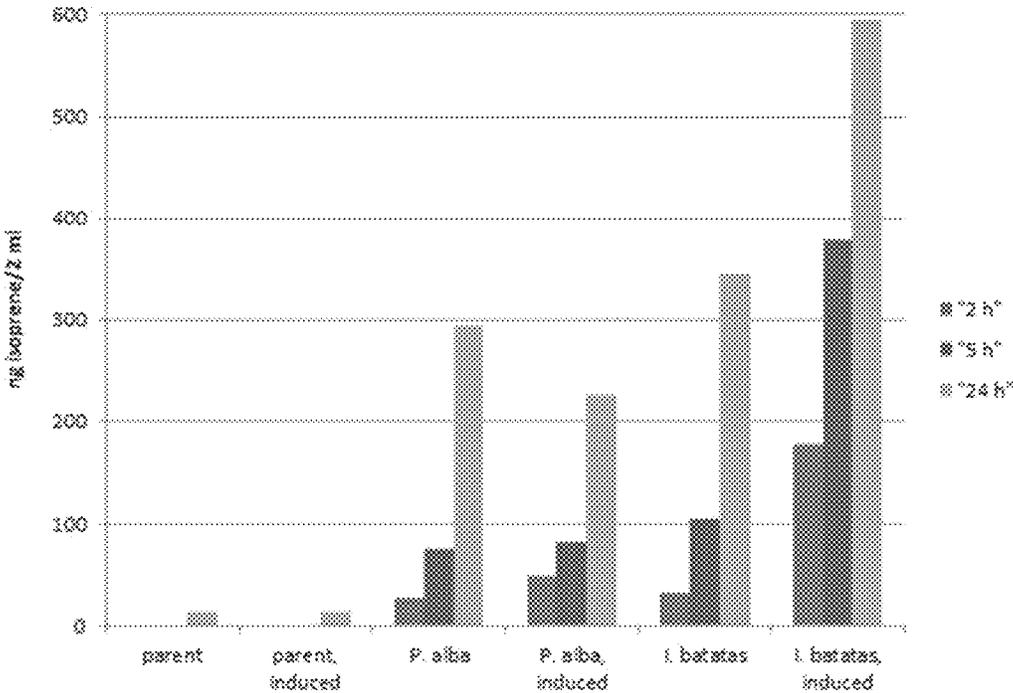


Fig. 12

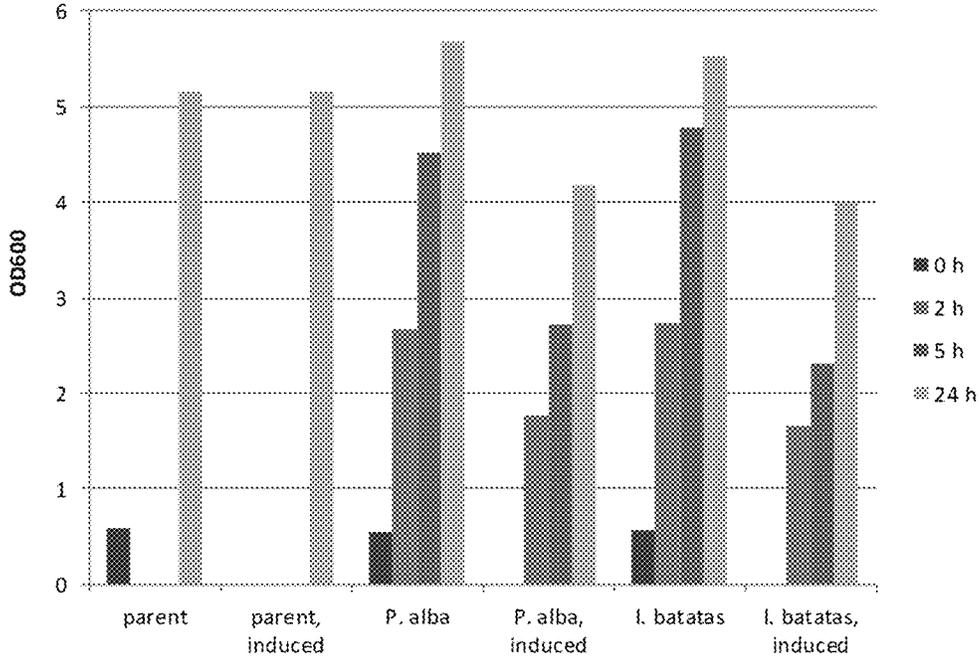


Fig. 13

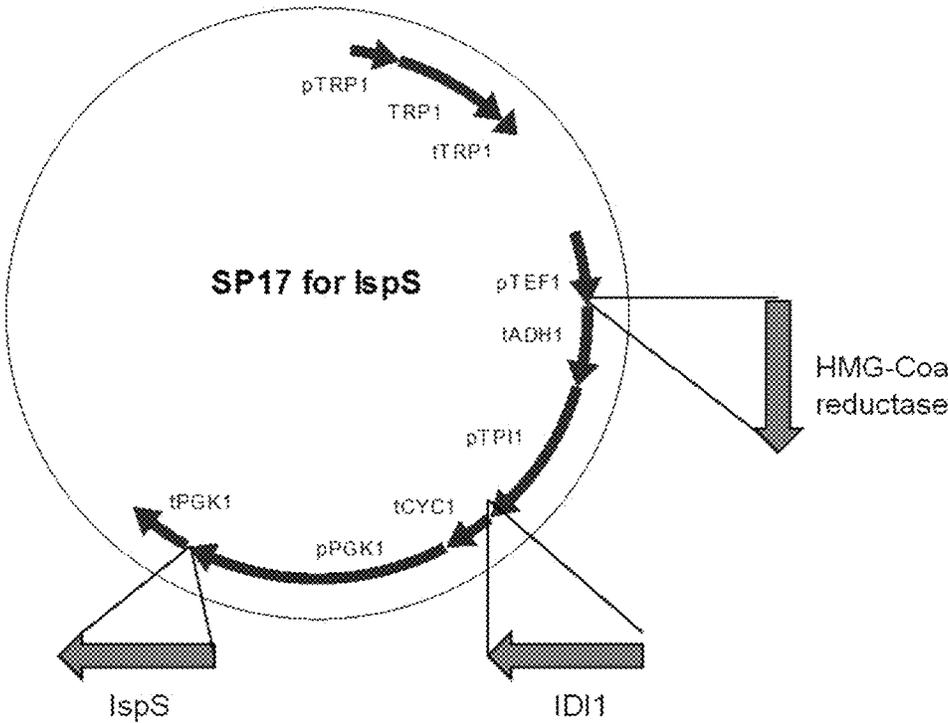


Fig. 14

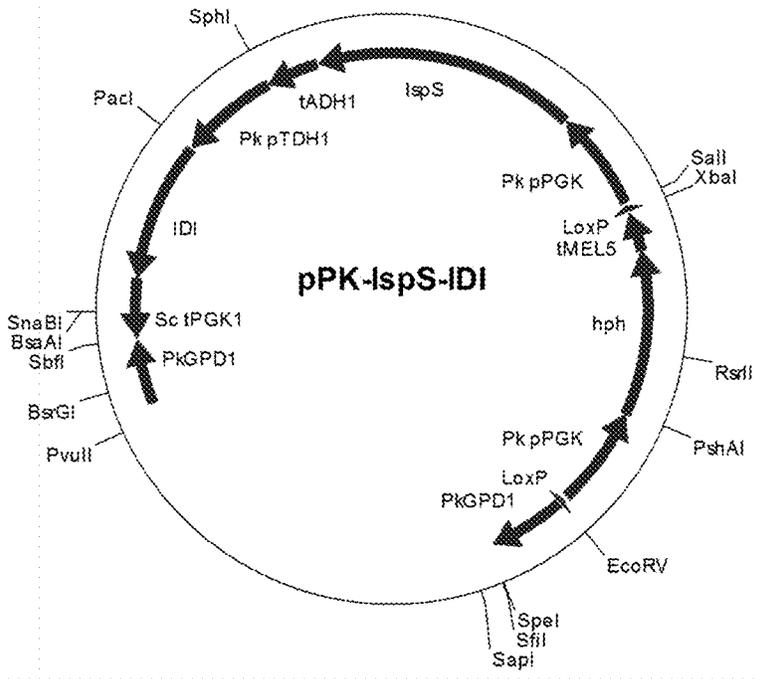


Fig. 15

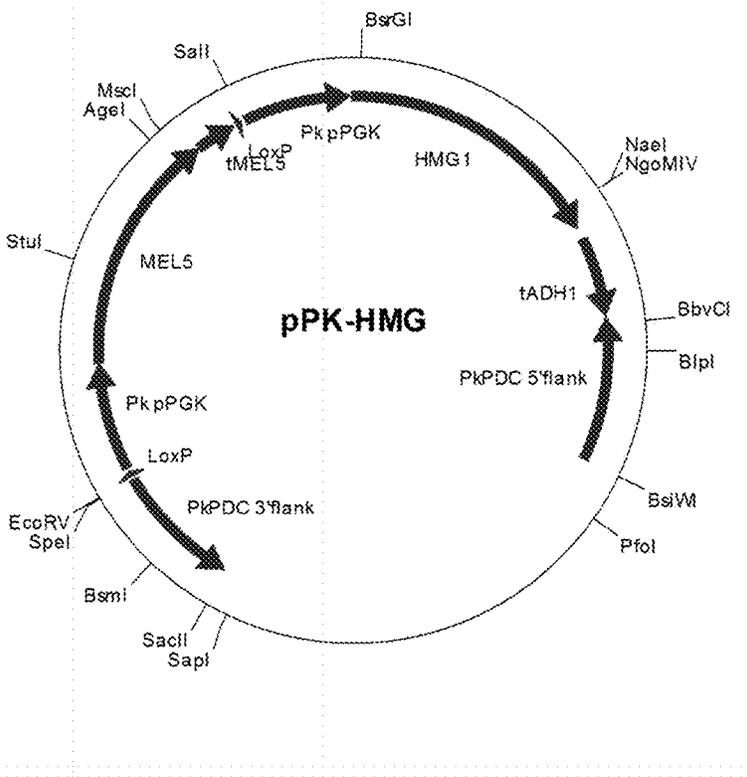


Fig. 16

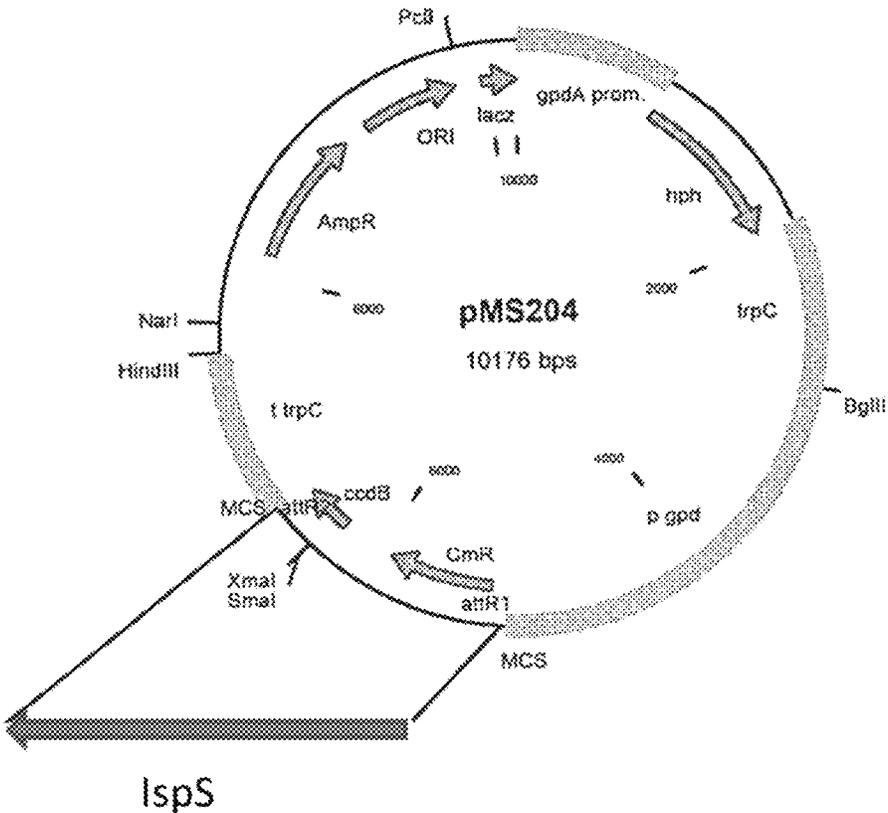


Fig. 17

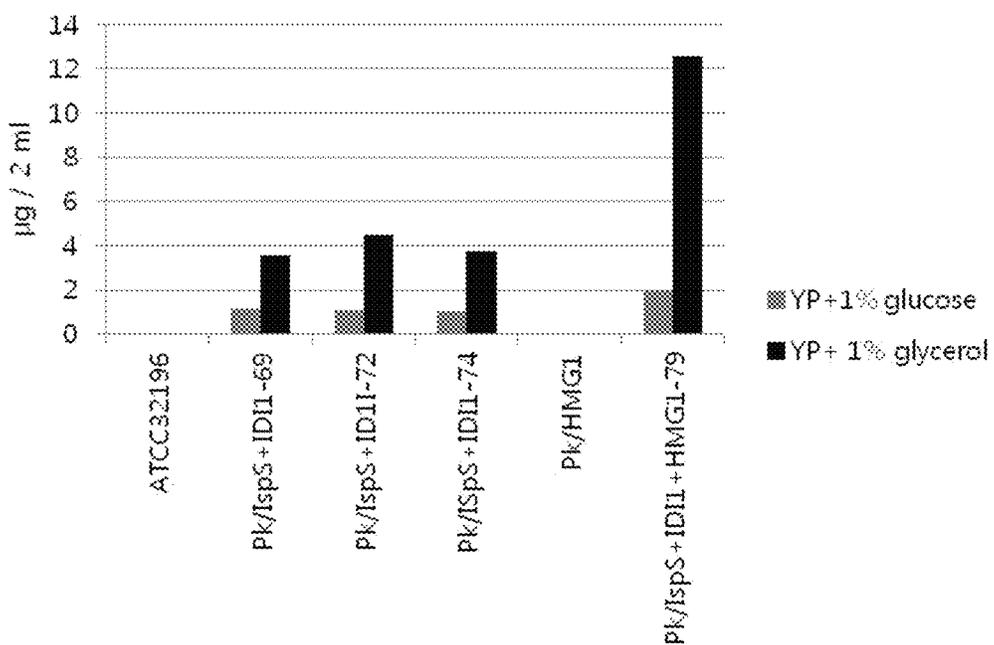


Fig. 18

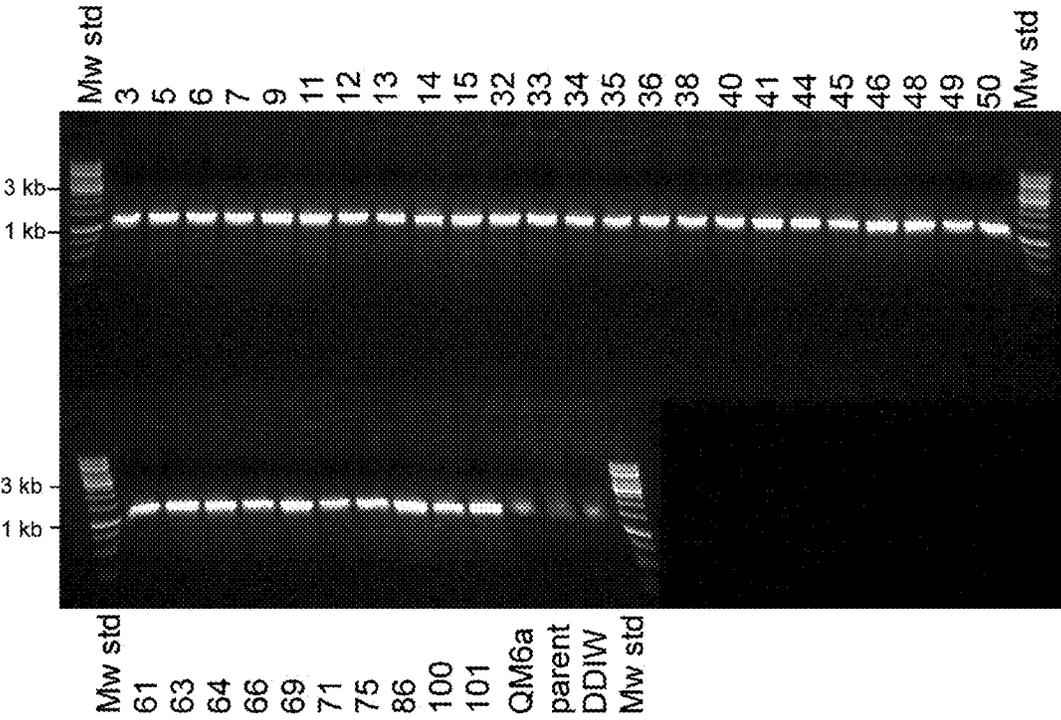


Fig. 20

ISOPRENE SYNTHASE AND METHOD OF PREPARING ISOPRENE USING THEREOF**CROSS-REFERENCE TO RELATED APPLICATIONS**

This application is a divisional application of U.S. patent application Ser. No. 15/329,343, filed Jan. 26, 2017, which was the United States national phase of International Application No. PCT/KR2015/007851 filed Jul. 28, 2015, which claims priority to Korean Patent Application No. 10-2014-0095972 filed Jul. 28, 2014, the disclosures of which are hereby incorporated in their entirety by reference.

The Sequence Listing associated with this application is filed in electronic format via EFS-Web and is hereby incorporated by reference into the specification in its entirety. The name of the text file containing the Sequence Listing is 1700617_ST25.txt. The size of the text file is 167,544 bytes, and the text file was created on Jan. 26, 2017.

TECHNICAL FIELD

The present invention relates to a novel isoprene synthase and a method of preparing isoprene using the same, and more specifically, to a polynucleotide encoding the novel isoprene synthase, a recombinant host cell having the polynucleotide introduced, and a method of preparing isoprene using the same.

BACKGROUND ART

Isoprenoids are isoprene polymers that find use in pharmaceuticals, nutraceuticals, flavors, fragrances, and rubber products. Supplies of natural isoprenoid, however, are restricted due to ecological concerns. For this reason, and in order to provide isoprenoid compositions having less impurities and greater uniformity, isoprenoids such as rubber are often produced synthetically. Isoprene (2-methyl-1,3-butadiene) is a volatile hydrocarbon that is insoluble in water and soluble in alcohol. Commercially viable quantities of isoprene can be obtained by direct isolation from petroleum C5 cracking fractions or by dehydration of C5 isoalkanes or isoalkenes (Weissermel and Arpe, *Industrial Organic Chemistry*, 4th ed., Wiley-VCH, pp. 117-122, 2003). The C5 skeleton can also be synthesized from smaller subunits.

It would be desirable, however, to have a commercially viable method of producing isoprene that was independent of nonrenewable resources. Biosynthetic production of isoprene occurs by two distinct metabolic pathways (Julsing et al., *Appl Microbiol Biotechnol*, 75:1377-1384, 2007). In eukaryotes and archae, isoprene is formed via the mevalonate (MVA) pathway, while some eubacteria and higher plants produce isoprene via the methylerythritol phosphate (MEP) pathway. Isoprene emissions from plants are light and temperature-dependent and increase with the association to leaf development.

An isoprene-producing enzyme, isoprene synthase, has been identified in Aspen trees (Silver and Fall, *Plant Physiol*, 97:1588-1591, 1991; and Silver and Fall, *J Biol Chem*, 270:13010-13016, 1995) and is believed to be responsible for the in vivo production of isoprene from whole leaves. Bacterial production of isoprene has also been described (Kuzma et al., *Curr Microbiol*, 30:97-103, 1995; and Wilkins, *Chemosphere*, 32:1427-1434, 1996), and it varies in amount according to the phase of bacterial growth and the

nutrient content of the culture medium (U.S. Pat. No. 5,849,970 to Fall et al.; and Wagner et al., *J Bacteriol*, 181:4700-4703, 1999).

The levels of isoprene obtainable through bacterial systems of the prior art, however, are insufficient for commercial uses. Thus, what the art needs is an effective and large scaled bacterial or microbial isoprene production process to provide feedstock for the manufacture of isoprene.

Accordingly, as a result of an effort for developing a method of preparing isoprene using a novel isoprene synthase gene having excellent isoprene productivity, the present inventors performed mining on a novel isoprene synthase gene, and confirmed that a recombinant microorganism transformed with the isoprene synthase gene has more excellent isoprene productivity than that of a host cell transformed with the isoprene synthase gene known in the art, thereby completing the present invention.

SUMMARY OF THE INVENTION**Technical Problem**

An object of the present invention is to provide an isoprene synthase having excellent isoprene productivity and a gene encoding the isoprene synthase.

Another object of the present invention is to provide a recombinant host cell expressing an isoprene synthase and a method of preparing isoprene by culturing the recombinant host cell.

Solution to Problem

In order to achieve the foregoing objects, the present invention provides an isoprene synthase comprising an amino acid sequence of SEQ ID NO: 1; or an amino acid sequence having 70% or more sequence homology to the amino acid sequence of SEQ ID NO: 1.

In addition, the present invention provides a polynucleotide encoding the isoprene synthase as described above and a recombinant vector into which the polynucleotide is operably introduced.

Further, the present invention provides a recombinant host cell into which the polynucleotide or the recombinant vector as described above is introduced.

In addition, the present invention provides a polynucleotide comprising a nucleotide sequence of SEQ ID NO: 3 optimized by codon optimization of the polynucleotide sequence encoding the isoprene synthase for blue-green algae (cyanobacteria); and a recombinant vector into which the polynucleotide is operably introduced.

In addition, the present invention provides a polynucleotide comprising a nucleotide sequence of SEQ ID NO: 33 optimized by codon optimization of the polynucleotide sequence encoding the isoprene synthase for yeast; and a recombinant vector into which the polynucleotide is operably introduced.

Further, the present invention provides recombinant blue-green alga (cyanobacteria) or recombinant filamentous fungi; into which the polynucleotide comprising the nucleic acid sequence of SEQ ID NO: 3 as described above or the recombinant vector comprising the polynucleotide as described above is introduced.

Further, the present invention provides recombinant yeast; into which the polynucleotide comprising the nucleic acid sequence of SEQ ID NO: 33 as described above or the recombinant vector comprising the polynucleotide as described above is introduced.

In addition, the present invention provides a method of preparing isoprene including: (a) culturing the recombinant host cell as described above to prepare isoprene; and (b) obtaining the prepared isoprene.

Further, the present invention provides a method of preparing isoprene including: (a) culturing the recombinant blue-green algae (cyanobacteria), filamentous fungi or yeast as described above to prepare isoprene; and (b) obtaining the prepared isoprene.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1a and FIG. 1b show an amino acid sequence alignment result of *I. batatas*—derived isoprene synthase with known isoprene synthases (SEQ ID NOS: 37-51).

FIG. 2 is directed to multiple sequence alignment (MSA) result showing the sequence around the substrate binding amino acids in isoprene synthase (SEQ ID NOS: 52-66).

FIG. 3 shows a multiple sequence alignment (MSA) result to around the substrate-binding amino acid sequence (SEQ ID NOS: 67-96).

FIG. 4 shows a phylogenetic tree among isoprene synthase candidates.

FIG. 5 shows a multiple sequence alignment (MSA) result of the isoprene synthase candidates.

FIG. 6 shows a map of pETDuet-1 vector.

FIG. 7 shows an expression of an isoprene synthase of isoprene synthase clone comprising a pBAT4 construct (trc promoter) of *P. alba*- and *I. batatas*-derived isoprene synthase genes.

FIG. 8 shows isoprene productivity of the pBAT4 construct (trc promoter) comprising *P. alba*- and *I. batatas*-derived isoprene synthase genes.

FIG. 9 shows an expression of an isoprene synthase of an *E. coli* strain transformed into a pETDuet-1 vector having five kinds of isoprene synthases (*I. batatas*, *E. photiniifolius*, *P. alba*, *P. montana* and *A. hypogaea*) introduced thereinto.

FIG. 10 shows isoprene productivity of an *E. coli* strain transformed into a pETDuet-1 vector, in which five kinds of isoprene synthases (*I. batatas*, *E. photiniifolius*, *P. alba*, *P. montana* and *A. hypogaea*) are inserted.

FIG. 11 shows inhibition in growth of an *E. coli* strain transformed into a pETDuet-1 vector, in which five kinds of isoprene synthases (*I. batatas*, *E. photiniifolius*, *P. alba*, *P. montana* and *A. hypogaea*) are inserted.

FIG. 12 shows time dependent isoprene productivity after expression of isoprene synthases in an *E. coli* strain transformed into a pETDuet-1 vector in which *I. batatas* of isoprene synthases and *P. alba* of isoprene synthases, respectively, are inserted.

FIG. 13 shows time dependent growth inhibition in of an *E. coli* strain transformed into a pETDuet-1 vector, in which *I. batatas* of isoprene synthases and *P. alba* of isoprene synthases, respectively, are inserted.

FIG. 14 shows plasmid for expression of IspS, IDI and HMG-CoA reductase in *S. cerevisiae*.

FIG. 15 shows plasmid for expression of IspS, and IDI in *P. kudriavzevii*.

FIG. 16 shows plasmids for expression of HMG-reductase in *P. kudriavzevii*.

FIG. 17 shows plasmid for expression of IspS in filamentous fungi.

FIG. 18 shows isoprene production by *P. kudriavzevii* transformants in YP medium containing 1% glucose (grey) or 1% glycerol (black) as the carbon source.

FIG. 19 shows plasmid pCIL105 for expression of *I. batatas* IspS in *T. reesei*.

FIG. 20 shows PCR analysis for the presence of the IspS expression cassette in *T. reesei* transformed with pCIL105. Wild type strain QM6a, parent and DDIW are negative controls.

DESCRIPTION OF THE INVENTION

Unless defined otherwise, all the technical and scientific terms used herein have the same meanings as those generally understood by persons skilled in the art to which the present invention pertains. Generally, the nomenclature used herein are well known and commonly employed in the art.

In a first aspect of the present invention, the present invention provides an isoprene synthase comprising an amino acid sequence of SEQ ID NO: 1 or an amino acid sequence having at least 70%, preferably at least 75%, more preferably at least 80%, still more preferably at least 85%, still more preferably at least 90%, still more preferably at least 95%, more and more preferably at least 98%, most preferably at least 99% sequence homology to the amino acid sequence of SEQ ID NO: 1.

The isoprene synthase belongs to a terpene synthase (TPS)-B family, and has an amino acid sequence specifically conserved in isoprene synthase, referred to as an “isoprene score” (Sharkey et al., *Evolution*, 67:1026, 2013). Important amino acids in the isoprene synthase are F338, 5445, F485 and N505 based on amino acid sequences of the *Populus alba*- and *Populus canescens*-derived isoprene synthases, and phenylalanine of F338 and F485 are important amino acids to decrease the size of a substrate-binding site so that large substrates such as geranyl diphosphate, farnesyl diphosphate, geranylgeranyl diphosphate, and the like, are not allowed to enter into an active site of an enzyme.

Therefore, the isoprene synthase of the present invention is characterized by comprising an amino acid sequence having 90% or more sequence homology to sequences at positions corresponding to RDRLMESFFW at position Nos. 257-266, FKLVTVLDDVYD at position Nos. 287-298, F369, SVS at position Nos. 394-396, FRLANDLSSS-KAEIERGETANSI at position Nos. 433-455, and YQYG-DAH at position Nos. 515-521 in an amino acid sequence of SEQ ID NO: 1. In addition, the isoprene synthase of the present invention is characterized by having at positions corresponding to the positions in SEQ ID NO: 1 at least one, preferably at least two, more preferably at least three, still more preferably at least four of the amino acids W266, F287, F369, F433, N453 and Y515.

The isoprene synthase of SEQ ID NO: 1 of the present invention comprises an amino acid sequence derived from sweet potato (*Ipomoea batatas*).

An amino acid sequence alignment result of *I. batatas*-derived isoprene synthase is shown in FIG. 1a and FIG. 1b, and an amino acid sequence alignment result of isoprene synthase candidates including *P. alba*-, *A. hypogaea*-derived isoprene synthases which are two known isoprene synthases, as a reference is shown in FIG. 2.

As a result of searching patent sequence database based on *I. batatas*-derived isoprene synthase having an amino acid sequence of SEQ ID NO: 1 according to the present invention, it was confirmed that the isoprene synthase derived from sweet potato of the present invention has 56% sequence homology to *Quercus petraea*-derived isoprene synthase (U.S. Patent Application Publication No. 2013/0330709) and has low sequence homology to the isoprene synthase known in the related art.

In a second aspect of the present invention, the present invention provides a polynucleotide encoding the isoprene synthase.

In the present invention, the polynucleotide may be codon-optimized for microorganisms selected from the group consisting of *E. coli*, *bacillus* genus strain, blue-green algae (cyanobacteria), yeast and filamentous fungi. Also the introns may be removed from the original gene. In the present invention the term "polynucleotide" is used to mean the gene in its original or modified form or the coding sequence in its original or modified form.

The codon optimization in the present invention means that a codon having an average codon frequency less than 12% on the microorganism is substituted with a codon having an average codon frequency more than 12% on the microorganism.

In an exemplary embodiment of the present invention, with a target of a isoprene synthase polynucleotide of SEQ ID NO: 2, the codon optimization was performed to enable expression in either *Synechocystis* sp. (strain PCC6803), which is blue-green algae cyanobacteria, or *E. coli* (SEQ ID NO: 3). This same codon optimized nucleotide sequence for bacteria can be used also for filamentous fungi. In an exemplary embodiment of the present invention SEQ ID NO:3 was used for the filamentous fungus *Trichoderma*.

In another exemplary embodiment of the present invention, with a target of an isoprene synthase polynucleotide of SEQ ID NO: 2, the codon usage was optimized for *S. cerevisiae* (SEQ ID NO:33) to enable expression either in *S. cerevisiae* or *Pichia* (*P. kudriavzevii*).

Therefore, in a preferred embodiment of the present disclosure, the polynucleotide encoding the isoprene synthase might comprise any one nucleotide sequence of SEQ ID NOs: 2, 3 or 33, but the present invention is not limited thereto.

In a third aspect of the present invention, the present invention provides a polynucleotide encoding an isoprene synthase comprising a nucleotide sequence of SEQ ID NO: 3 by codon optimization of the polynucleotide encoding the isoprene synthase for blue-green algae (cyanobacteria), and *E. coli*, useful also for other bacteria and filamentous fungi, in particular *Trichoderma* and *Aspergillus*.

In a fourth aspect of the present invention, the present invention provides a polynucleotide encoding an isoprene synthase comprising a nucleotide sequence of SEQ ID NO: 33 by codon optimization of the polynucleotide encoding the isoprene synthase for *Saccharomyces cerevisiae*, useful also for *Saccharomyces*, *Pichia* and other yeasts.

In a fifth aspect of the present invention, the present invention provides a recombinant vector into which the polynucleotide is operably introduced; and a recombinant host cell into which the polynucleotide or the recombinant vector is introduced.

The recombinant vector in the present invention may contain a promoter for expressing the polynucleotide.

Examples of the promoter contained in the recombinant vector could include psbA2, trc, rbcL, petJ, psaA, psaB, tac, cpcB, petC or lac promoters for expression in blue-green algae (cyanobacteria), PGK1, TPI1, TDH, PDC1, FBA1, ENO1, ENO2, PYK1, ADH1, or TEF1 promoters for expression in yeast, preferably the promoter is selected from the group consisting of PGK1, TPI1 and TEF1 for expressing in yeast., cbh1, gpdA, glaA, pdc or exlA promoters for expression in filamentous fungi, and a CMV35S promoter for expression in a plant cell, but the present invention is not limited thereto.

In a preferred embodiment of the present invention, a polynucleotide sequence is contained in plasmids. In another preferred embodiment of the present invention, a polynucleotide sequence is incorporated into the genome of a host cell. In some preferred embodiments of the present invention, a host is selected from the group consisting of gram-positive bacterial cells, gram-negative bacterial cells, filamentous fungal cells, and yeast cells, but the present invention is not limited thereto.

In some preferred embodiments of the present invention, *escherichia* species (*E. coli*), *pantoea* species (*Pantoea citrea*), *bacillus* species (*Bacillus subtilis*), *yarrowia* species (*Yarrowia lipolytica*), and *trichoderma* species (*Trichoderma reesei*), but the present invention is not limited thereto. In some preferred embodiments, the host cell is cultured in a medium containing carbon sources selected from the group consisting of CO₂, bicarbonate, glucose, glycerol, glycerine, dihydroxyacetone, yeast extract, biomass, molasses, sucrose, galactose, sorbose, sorbitol, xylose, arabinose, cellulose, xylan, lactose and oil, but not limited thereto.

Preferably, the host cell of the present invention may be blue-green algae (cyanobacteria), yeast, filamentous fungi, or plant cells.

In a preferred embodiment of the present invention, the filamentous fungi may be *Trichoderma* genus, *Mucor* genus, *Mortierella* genus, *Neurospora* genus or *Aspergillus* genus, advantageously the filamentous fungi is *Trichoderma* or *Aspergillus* genus and the plant cell may be *Nicotiana* genus, *Catharantus* genus or *Hyrosocyamus* genus plant cells.

In a sixth aspect of the present invention, the present invention provides a polynucleotide comprising a nucleotide sequence of SEQ ID NO: 3 by codon optimization of the polynucleotide encoding the isoprene synthase on blue-green algae (cyanobacteria), useful also for other bacteria and filamentous fungi, and a recombinant vector into which the polynucleotide is operably introduced; and a recombinant blue-green algae (cyanobacteria), bacteria or filamentous fungi into which the polynucleotide or the recombinant vector is introduced.

In a seventh aspect of the present invention, the present invention provides a polynucleotide comprising a nucleotide sequence of SEQ ID NO:33 by codon optimization of the polynucleotide encoding the isoprene synthase on *Saccharomyces cerevisiae* useful for *Saccharomyces*, *Pichia* and other yeasts, and a recombinant vector into which the polynucleotide is operably introduced; and a recombinant yeast into which the polynucleotide or the recombinant vector is introduced.

In the present invention, the recombinant vector may comprise psbA2, trc, rbcL, petJ, psaA, psaB, tac, cpcB, petC or lac promoter for expression in blue-green algae (cyanobacteria).

Preferably, the blue-green algae may be unicellular blue-green algae or multicellular blue-green algae. The unicellular blue-green algae may be *Synechocystis* genus or *Synechococcus* genus strain, and the multicellular blue-green algae may be *Gloeocapsa* genus or filamentous cyanobacteria.

In a preferred embodiment of the present invention, the filamentous cyanobacteria may be *Nostoc* genus, *Anabaena* genus or *Arthospira* genus, and the yeast may be *Saccharomyces* genus, *Pichia* genus, *Candida* genus, *Kazachstania* genus, *Kluyveromyces* genus, *Hansenula* genus, *Rhodospiridium* genus, *Cryptococcus* genus or *Yarrowia* genus.

The polynucleotide is operably linked when being disposed with other nucleic acid sequences in a functional

relationship. This may be polynucleotide and a regulatory sequence(s) linked in the manner of enabling polynucleotide expression when appropriate molecules (for example, transcriptional activation protein) are bound to the regulatory sequence(s). For example, nucleotide sequence for a pre-sequence or a secretion leader is operably linked to nucleotide sequence for a polypeptide when being expressed as a pre-protein participating in secretion of polypeptide; a promoter or an enhancer is operably linked to a coding sequence when having an influence on transcription of sequence; or a ribosome binding site is operably linked to a coding sequence when having an influence on transcription of sequence; or a ribosome binding site is operably linked to a coding sequence when being disposed so that translation is easily performed. In general, term: 'operably linked' means that the linked nucleotide sequences are contacted with each other, or in the case of the secretion leader, contacted with the nucleotide sequence and present within a leading frame. However, the enhancer does not need to have a contact. The link of the sequences is performed by ligation (linkage) in a convenient restriction enzyme site. When the site does not exist, a synthetic oligonucleotide adaptor or a linker according to general method is used.

A method of inserting the polynucleotide into the genome of a host cell may be a generally known genetic engineering method, and a non-viral transfer method may include electroporation, lipofection, microinjection, biolistic, virosome, liposome, immuno-liposome, multivalent cation or lipid: nucleic acid conjugate, naked DNA, artificial virion and chemical-enhanced uptake of DNA. Sonoporation, for example, a method using a sonitron 2000 system (Rich-Mar) may be used for transfer of nucleic acids, and other representative nucleic acid transfer systems include Amaxa Biosystems (Cologne, Germany), Maxcyte, Inc. (Rockville, Md.) and BTX Molecular System (Holliston, Mass.). The lipofection method is disclosed in U.S. Pat. Nos. 5,049,386, 4,946,787, and 4,897,355, and lipofection reagent is commercially available, for example, Transfectam™ and Lipofectin™. Cationic and neutral lipids that are suitable for efficient receptor-recognition lipofection of polynucleotides include those of Feigner (WO 91/17424 and WO 91/16024), and may be transferred to cells via ex-vivo administration or to target tissues via in-vivo administration. A method of preparing lipid:nucleic acid complex, containing targeted liposomes such as immunolipid complexes, is well known in the art (Crystal, *Science*, 270:404-410, 1995; Blaese et al., *Cancer Gene Ther.*, 2:291-297, 1995; Behr et al., *Bioconjugate Chem.*, 5:382389, 1994; Remy et al., *Bioconjugate Chem.*, 5:647-654, 1994; Gao et al., *Gene Therapy*, 2:710-722, 1995; Ahmad et al., *Cancer Res.*, 52:4817-4820, 1992; U.S. Pat. Nos. 4,186,183; 4,217,344; 4,235,871; 4,261,975; 4,485,054; 4,501,728; 4,774,085; 4,837,028; 4,946,787).

The tropism of a retrovirus may be altered by unification with foreign envelope proteins and thereby expand kinds of target cells. A lentiviral vector is a type of retroviral vector that is able to transduce or infect non-dividing cells and produce high viral titers. A retroviral gene transfer system is determined depends on the target tissue. The retroviral vector includes cis-acting long terminal repeats with packaging capacity for 6-10 kb of foreign sequence. The minimum cis-acting LTRs which are sufficient for replication and packaging of the vectors may be used to integrate the therapeutic gene into the target cell to provide permanent transgene expression. Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immunodeficiency virus (SIV), human immunodeficiency virus (HIV), and combi-

nations thereof (Buchscher et al., *J. Virol.*, 66:2731-2739, 1992; Johann et al., *J. Virol.*, 66:1635-1640, 1992; Sommerfelt et al., *Virol.*, 176:58-59, 1990; Wilson et al., *J. Virol.*, 63:2374-2378, 1989; Miller et al., *J. Virol.*, 65:2220-2224, 1991).

In a case of temporarily expressing a sucrose phosphorylase protein, an adenoviral based system may be frequently used. Adenoviral based vectors are capable of very high transduction efficiency in many cell types and do not require cell division. When using the vectors, high titer and high levels of expression may be obtained and a mass-production is possible with a relatively simple system. In addition, Adeno-associated virus ("AAV") vectors are also used to transduce cells with target nucleic acids, for example, for in vitro production of nucleic acids and peptides, and for in vivo and ex vivo gene therapy (West et al., *Virology*, 160:38-47, 1987; U.S. Pat. No. 4,797,368; WO 93/24641; Kotin, *Human Gene Therapy*, 5:793-801, 1994; Muzyczka, *J. Clin. Invest.*, 94:1351, 1994), and a construction of recombinant AAV vectors were already known (U.S. Pat. No. 5,173,414; Tratschin et al., *Mol. Cell. Biol.*, 5:3251-3260, 1985; Tratschin, et al., *Mol. Cell. Biol.*, 4:20722081, 1984; Hermonat & Muzyczka, *PNAS*, 81:6466-6470, 1984; Samulski et al., *J. Virol.*, 63:3822-3828, 1989). In clinical trials, at least six viral vector approaches are currently available for gene transfer, which utilize approaches that involve complementation of defective vectors by genes inserted into helper cell lines to generate the transducing agent. pLASN and MFG-S are examples of retroviral vectors that have been used in clinical trials (Dunbar et al., *Blood*, 85:3048, 1995; Kohn et al., *Nat. Med.*, 1:1017, 1995; Malech et al., *PNAS*, 94:12133, 1997), and PA317/pLASN is the first therapeutic vector used in a gene therapy trial (Blaese et al., *Science*, 270:475-480, 1995), and transduction efficiencies of 50% or greater have been observed for MFG-S packaged vectors (Ellem et al., *Immunol Immunother.*, 44(1):10-20, 1997; Dranoff et al., *Hum. Gene Ther.*, 1:111-2, 1997).

Recombinant adeno-associated virus vectors (rAAV) are a promising alternative gene delivery systems based on the defective and nonpathogenic parvovirus adeno-associated type 2 virus. All vectors are derived from a plasmid that retains the AAV 145 bp inverted terminal repeats flanking the transgene expression cassette. Efficient gene transfer and stable transgene delivery due to integration into the genomes of the transduced cell are key features for this vector system (Wagner et al., *Lancet*, 351:9117, 1998; Kearns et al., *Gene Ther.*, 9:748-55, 1996).

In an eighth aspect of the present invention, the present invention provides a method of preparing isoprene including: (a) culturing the recombinant host cell as described above to prepare isoprene; and (b) obtaining the prepared isoprene.

The culturing of the step (a) may be performed in a medium containing carbon sources selected from the group consisting of CO₂, bicarbonate, glucose, glycerol, glycerine, dihydroxyacetone, yeast extract, biomass, molasses, sucrose, galactose, sorbose, sorbitol, xylose, arabinose, cellulose, xylan, lactose and oil.

In a ninth aspect of the present invention, the present invention provides a method of preparing isoprene including: (a) culturing the recombinant blue-green algae (cyanobacteria), bacteria, recombinant filamentous fungi or yeast as described above to prepare isoprene; and (b) obtaining the prepared isoprene.

The methods may additionally include (c) polymerizing isoprene.

Unless otherwise indicated, the practice of the present invention involves conventional techniques commonly used in molecular biology, microbiology, and recombinant DNA, which are within the skill of the art. Such techniques are known to those of skill in the art and are described in numerous texts and reference works (See e.g., Sambrook et al., "Molecular Cloning: A Laboratory Manual," Second Edition, Cold Spring Harbor, 1989; and Ausubel et al., "Current Protocols in Molecular Biology," 1987).

Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. For example, Singleton and Sainsbury, Dictionary of Microbiology and Molecular Biology, 2d Ed., John Wiley and Sons, NY (1994); and Hale and Marham, The Harper Collins Dictionary of Biology, Harper Perennial, N.Y. (1991) provides those of skill in the art with a general dictionary of many of the terms used in the invention. Although any methods and materials similar or equivalent to those described herein find use in the practice of the present invention, the preferred methods and materials are described herein. Accordingly, the terms defined immediately below are more fully described by reference to the Specification as a whole.

In combination with isoprene synthase expression of other genes contributing to isoprene production can be introduced into blue green algae (cyanobacteria), bacteria, filamentous fungi or yeast, cells. For example, the *idi* gene coding for isopentenyl-diphosphate delta-isomerase (EC 5.3.3.2) can be introduced to enhance conversion of isopentenyl diphosphate (IPP) to dimethylallyl diphosphate (DMAPP) that is the substrate of isoprene synthase. Also one or more genes coding for the mevalonate pathway components, mevalonate kinase (EC2.7.1.36), phosphomevalonate kinase (EC2.7.4.2), pyrophosphomevalonate decarboxylase (EC 4.1.1.33), acetoacetyl-CoA thiolase (EC2.3.1.9), HMG-CoA synthase (EC2.3.3.10) or HMG-CoA reductase (EC1.1.1.34) can be cloned under a suitable cyanobacteria, bacteria, filamentous fungi or yeast promoter that allow expression in these hosts. Two or more genes are expressed as a transcriptional fusion under the control or under the transcriptional control of a cyanobacteria or bacteria promoter. When two or more genes are expressed in, filamentous fungi or yeast, each gene is expressed under the control of an individual filamentous fungal or yeast promoter. Advantageous for expression of isoprene synthase is for example a combination of *IDI* and HMG reductase in filamentous fungi and in yeast, in particular in *Pichia* and *Saccharomyces*.

As used herein, the term 2-methyl-1,3-butadiene (CAS #78-79-5) ("isoprene") refers to the direct and final volatile C5 hydrocarbon product from the elimination of pyrophosphate from 3,3-dimethylallyl pyrophosphate (DMAPP), and does not involve the linking or polymerization of [an] IPP molecule(s) to [a] DMAPP molecule(s).

As used herein, the terms "isoprene synthase," and "IspS," refer to the enzymes that catalyze the elimination or pyrophosphate from diemethylallyl diphosphate (DMAPP) to form isoprene.

In some embodiments, the term "IspS" refers to a naturally occurring mature enzyme or portion thereof.

The present invention comprises proteins comprising an amino acid sequence having 70% or more identity with the amino acid sequence of Isoprene synthase from sweet potato, the proteins comprise "variant proteins".

In some preferred embodiments, variant proteins differ from a parent protein (e.g., set forth as SEQ ID NO:1) by a small number of amino acid residues. The number of dif-

fering amino acid residues may be one or more, preferably 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, or more amino acid residues. In some preferred embodiments, the number of different amino acids between variants is between 1 and 10.

In some particularly preferred embodiments, related proteins and particularly variant proteins comprise at least 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% amino acid sequence identity. Additionally, a related protein or a variant protein as used herein refers to a protein that differs from another related protein or a parent protein in the number of prominent regions. For example, in some embodiments, variant proteins have 1, 2, 3, 4, 5, or 10 corresponding prominent regions that differ from the parent protein.

In present invention, the proteins comprising an amino acid sequence having 70% identity with amino acid sequence set forth as SEQ ID NO:1 of Isoprene synthase can be generated with several methods including but not limited to site-saturation mutagenesis, scanning mutagenesis, insertional mutagenesis, random mutagenesis, site-directed mutagenesis, and directed-evolution, as well as various other recombinatorial approaches.

As used herein the term "gene" refers to a polynucleotide (e.g., a DNA segment) that encodes a polypeptide and includes regions preceding and following the coding regions as well as intervening sequences (introns) between individual coding segments (exons). Polynucleotides introduced into host cells may comprise the coding region without introns and with or without the preceding or following regions of the original gene. Furthermore the codons may be modified to be more suitable for the host cell.

As used herein, "homology" refers to sequence similarity or identity, with identity being preferred. This homology is determined using standard techniques known in the art (See e.g., Smith and Waterman, Adv Appl Math, 2:482, 1981; Needleman and Wunsch, J Mol Biol, 48:443, 1970; Pearson and Lipman, Proc Natl Acad Sci USA, 85:2444, 1988; programs such as GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, Madison, Wis.; and Devereux et al., Nucl Acid Res, 12:387-395, 1984).

As used herein, an "analogous sequence" is one wherein the function of the polynucleotide is essentially the same as the polynucleotide based on sweet potato isoprene synthase (IspS). Additionally, analogous polynucleotides include at least 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% sequence identity with the amino acid sequence of the batate isoprene synthase. In additional embodiments more than one of the above properties applies to the sequence. Analogous sequences are determined by known methods of sequence alignment. A commonly used alignment method is BLAST, although as indicated above and below, there are other methods that also find use in aligning sequences.

Thus, "percent (%) nucleic acid sequence identity" is defined as the percentage of nucleotide residues in a candidate sequence that is identical to the nucleotide residues of the starting sequence (i.e., the sequence of interest). A preferred method utilizes the BLASTN module of WU-BLAST-2 set to the default parameters, with overlap span and overlap fraction set to 1 and 0.125, respectively.

As used herein, "recombinant" includes reference to a cell or vector, that has been modified by the introduction of a heterologous nucleic acid sequence or that the cell is derived from a cell so modified. Thus, for example, recombinant cells expresses genes that are not found in identical form within the native (non-recombinant) form of the cell or

express native genes that are otherwise abnormally expressed, under expressed or not expressed at all as a result of deliberate human intervention. "Recombination," "recombining," and generating a "recombined" nucleic acid are generally the assembly of two or more nucleic acid fragments wherein the assembly gives rise to a chimeric gene.

As used herein, the terms "amplification" and "gene amplification" refer to a process by which specific polynucleotides are disproportionately replicated such that the amplified polynucleotide becomes present in a higher copy number than was initially present in the genome. In some embodiments, selection of cells by growth in the presence of a drug (e.g., an inhibitor of an inhibitable enzyme) results in the amplification of either the endogenous gene encoding the gene product required for growth in the presence of the drug or by amplification of exogenous (i.e., input) sequences encoding this gene product, or both.

"Amplification" is a special case of nucleic acid replication involving template specificity. It is to be contrasted with non-specific template replication (i.e., replication that is template-dependent but not dependent on a specific template). Template specificity is here distinguished from fidelity of replication (i.e., synthesis of the proper polynucleotide sequence) and nucleotide (ribo- or deoxyribo-) specificity. Template specificity is frequently described in terms of "target" specificity. Target sequences are "targets" in the sense that they are sought to be sorted out from other nucleic acid. Amplification techniques have been designed primarily for this sorting out.

As used herein, the terms "amplifiable marker," "amplifiable gene," and "amplification vector" refer to a polynucleotide or a vector comprising a polynucleotide, which permits the amplification of that polynucleotide under appropriate growth conditions.

"Homologous sequences" as used herein means a nucleic acid or polypeptide sequence having 100%, 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 88%, 85%, 80%, 75%, or 70% sequence identity to another nucleic acid or polypeptide sequence when optimally aligned for comparison. In some embodiments, homologous sequences have between 85% and 100% sequence identity, while in other embodiments there is between 90% and 100% sequence identity, and in more preferred embodiments, there is 95% and 100% sequence identity.

As used herein "amino acid" refers to peptide or protein sequences or portions thereof. The terms "protein," "peptide," and "polypeptide" are used interchangeably.

As used herein, the term "heterologous protein" refers to a protein or polypeptide that does not naturally occur in the host cell. Examples of heterologous proteins include enzymes such as isoprene synthases. In some embodiments, the polynucleotides encoding the proteins are naturally occurring genes, while in other embodiments mutated and/or synthetic polynucleotides are used.

As used herein, "homologous protein" refers to a protein or polypeptide native or naturally occurring in a cell. In preferred embodiments, the cell is a Gram-negative cell, while in particularly preferred embodiments the cell is an *Escherichia* host cell.

An enzyme is "overexpressed" in a host cell if the enzyme is expressed in the cell at a higher level than the level at which it is expressed in a corresponding wild-type cell.

The terms "protein" and "polypeptide" are used interchangeably herein. The 3-letter code for amino acids as defined in conformity with the IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN) is used through

out this disclosure. It is also understood that a polypeptide may be coded for by more than one nucleotide sequence due to the degeneracy of the genetic code.

The term "mature" form of a protein or peptide refers to the final functional form of the protein or peptide. To exemplify, a mature form of sweet potato isoprene synthase includes the amino acid sequence of SEQ ID NO: 1.

The term "precursor" form of a protein or peptide refers to a mature form of the protein having a prosequence operably linked to the amino or carboxyl terminus of the protein. The precursor may also have a "signal sequence" operably linked, to the amino terminus of the prosequence. The precursor may also have additional polynucleotides that are involved in post-translational activity (e.g., polynucleotides cleaved therefrom to leave the mature form of a protein or peptide).

"Naturally occurring enzyme" refers to an enzyme having the unmodified amino acid sequence identical to that found in nature. Naturally occurring enzymes include native enzymes, those enzymes naturally expressed or found in the particular microorganism.

The term "identical" in the context of two nucleic acids or polypeptide sequences refers to the residues in the two sequences that are the same when aligned for maximum correspondence, as measured using one of the following sequence comparison or analysis algorithms.

The term "optimal alignment" refers to the alignment giving the highest percent identity score. "Percent sequence identity," "percent amino acid sequence identity," "percent gene sequence identity," and/or "percent nucleic acid/polynucleotide sequence identity," with respect to two amino acid, polynucleotide and/or gene sequences (as appropriate), refer to the percentage of residues that are identical in the two sequences when the sequences are optimally aligned. Thus, 80% amino acid sequence identity means that 80% of the amino acids in two optimally aligned polypeptide sequences are identical.

The phrase "substantially identical" in the context of two nucleic acids or polypeptides thus refers to a polynucleotide or polypeptide that comprising at least 70% sequence identity, preferably at least 75%, preferably at least 80%, preferably at least 85%, preferably at least 90%, preferably at least 95%, preferably at least 97%, preferably at least 98% and preferably at least 99% sequence identity as compared to a reference sequence using the programs or algorithms (e.g., BLAST, ALIGN, CLUSTAL) using standard parameters. One indication that two polypeptides are substantially identical is that the first polypeptide is immunologically cross-reactive with the second polypeptide. Typically, polypeptides that differ by conservative amino acid substitutions are immunologically cross-reactive. Thus, a polypeptide is substantially identical to a second polypeptide, for example, where the two peptides differ only by a conservative substitution. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions (e.g., within a range of medium to high stringency).

As used herein, "corresponding to," refers to a residue at the enumerated position in a protein or peptide, or a residue that is analogous, homologous, or equivalent to an enumerated residue in a protein or peptide. As used herein, "corresponding region," generally refers to an analogous position along related proteins or a parent protein.

As used herein, the terms "multiple sequence alignment" and "MSA" refer to the sequences of multiple homologs of a starting gene that are aligned using an algorithm (e.g., Clustal W).

As used herein, the terms “consensus sequence” and “canonical sequence” refer to an archetypical amino acid sequence against which all variants of a particular protein or sequence of interest are compared. The terms also refer to a sequence that sets forth the nucleotides that are most often present in a DNA sequence of interest. For each position of a gene, the consensus sequence gives the amino acid that is most abundant in that position in the MSA.

As used herein, the term “consensus mutation” refers to a difference in the sequence of a starting gene and a consensus sequence. Consensus mutations are identified by comparing the sequences of the starting gene and the consensus sequence obtained from a MSA. In some embodiments, consensus mutations are introduced into the starting gene such that it becomes more similar to the consensus sequence. Consensus mutations also include amino acid changes that change an amino acid in a starting gene to an amino acid that is more frequently found in an MSA at that position relative to the frequency of that amino acid in the starting gene. Thus, the term consensus mutation comprises all single amino acid changes that replace an amino acid of the starting gene with an amino acid that is more abundant than the amino acid in the MSA.

As used herein, the term “headspace” refers to the vapor/air mixture trapped above a solid or liquid sample in a sealed vessel.

Unless otherwise noted, all component or composition levels are in reference to the active level of that component or composition, and are exclusive of impurities, for example, residual solvents or by-products, which may be present in commercially available sources.

Enzyme components weights are based on total active protein. All percentages and ratios are calculated by weight unless otherwise indicated. All percentages and ratios are calculated based on the total composition unless otherwise.

EXAMPLES

Hereinafter, the present invention will be described in detail with reference to the following Examples. However, the following examples are only for exemplifying the present invention and it will be obvious to those skilled in the art that the scope of the present invention is not construed to be limited to these examples.

Example 1: Genome Mining for Novel Isoprene Synthase Genetic Search

The isoprene synthase belongs to a terpene synthase (TPS)-B family, and has an amino acid specifically conserved in isoprene synthase, referred to as an “isoprene score” (Sharkey et al., *Evolution*, 67: 1026, 2013). Important amino acids in the isoprene synthase are F338, S445, F485 and N505 based on amino acid numbering of sequences of the *Populus alba*- and *Populus canescens*-derived isoprene synthases, and phenylalanines of F338 and F485 respectively are amino acids which have an important role to decrease a size of a substrate-binding site so that large substrates such as geranyl diphosphate, farnesyl diphosphate, geranylgeranyl diphosphate, and the like, are not allowed to enter into an active site of an enzyme.

Bi-functional myrcene synthase of *Humulus lupulus* having low activity as compared to other isoprene synthases lacks F485. N505 has a critical role to determine an ion requirement of terpene synthases, and terpene synthase without an ion requirement has cationic lysine, serine, or asparagine positioned at position No. 505. S445 is present at

a first position of triple serine motif, and other TPS-b protein usually has valine (Val) and isoleucine (Ile) in the middle of the triple serine motif; however, S445 present at the first position of the triple serine motif is conserved in almost all Tps-b family and thus, it is judged that S446 positioned in the middle of the triple serine motif is not important in preparation of isoprene.

In addition, W317 and Y565, which are amino acids present in almost all TPS family sequences, are essential to define a size of a substrate-bound pocket.

In addition, N489 is conserved in isoprene/ocimene clade of the Tps-B

In order to determine isoprene Synthase candidates, a homology-based database search was conducted. Isoprene synthase sequences using Query sequence are as follows:

- 1) *Populus alba* (white poplar, Uniprot: Q50L36), *Populus canescens* (grey poplar, Uniprot: Q9AR86, PDB: 3N0G) and *Pueraria montana* var. *lobata* (kudzu wine, Uniprot: Q6EJ97);
- 2) *Arachis hypogaea* (peanut, SEQ ID No: 3), Glycine max (soybean, SEQ ID No:5 and SEQ ID No:7), *Mucuna pruriens* (velvet bean, SEQ ID No:9), *Cajanus cajan* (pigeon pea, SEQ ID No:11) and *Quercus petraea* (oak, SEQ ID No:13) derived from WO 2013/166,320A1;
- 3) *Wisteria* sp. (GenBank: AEK70969), *Robinia pseudo-acacia* (GenBank: AEK70968), *Melaleuca alternifolia* (GenBank: AAP40638), *Eucalyptus globulus* (GenBank: BAF02831), *Salix* sp. (GenBank: AEK70970) and a myrcene synthase from *Humulus lupulus* (GenBank: ACI32638) derived from Sharkey list (Sharkey et al., *Evolution*, 67: 1026, 2013); and
- 4) 2-methyl-3-buten-2-ol (MBO) synthase (GenBank: AEB53064)(Gray et al., *J Biol Chem*, 286: 20582, 2011) derived from *Pinus sabiniana*.

In addition, several sequences derived from poplar genus were included as a reference.

The homology-based search was performed in GenBank protein databases (nr, pat and env_nr) using Uniprot(SwissProt and TrEMBL) and blastp, and was performed in GenBank nucleotide databases (tsa_nt, env_nt and pat) using tblastn, and extracted when E-value is less than 1e-30.

Additionally, Uniprot/SwissProt sequences with InterPro domain annotation of “Terpene synthase, metal binding domain” protein family (PFAM: PF03936, Interpro: IPR005630) were retrieved.

The searched nucleotide sequence was translated into an amino acid sequence by GeneWise program.

tsa_nt (GenBank TSA1) which is a database including cDNA sequence through Transcriptomic study was included in Query database, and in order to increase a usable coverage of a plant genome data, transcriptomic data on plant genomes of which sequences are determined up to date was included.

Accordingly, total 9123 sequences were searched, wherein 278 sequences were searched in Uniprot/SwissProt, 1,989 sequences were searched in Uniprot/TrEMBL, 3,953 sequences were searched in GenBank nucleotide databases, and 2,905 sequences were searched in GenBank protein databases. In order to remove the repeated sequences, sequences having 80% homology with each other clustered.

In order to confirm functional diversity among the searched terpene synthases, multiple sequence alignment (MAS) and phylogenetic tree were made by using script in a unix environment. Sequences were aligned with respect to

PFAM domain (PF03936) of a protein family and phylogenetic tree was made based on the MSA using FASTTREE program.

The active site of the isoprene synthase determined based on the structure of *Populus canescens* IpsS (PDB:3n0g) was marked to the MSA. The sequences were aligned by ClustalW, and a conserved phylogenetic tree was constructed with resampling using Genious tree builder.

As the final candidates of the plant-derived sequences, *Ipomoea batatas*-derived sequence and *Elaeocarpus photiniifolius*-derived sequence were determined.

Ipomoea batatas sequence has the key amino acids of isoprene synthases W317 (W266 in *I. batatas*), F338 (F287 in *I. batatas*), 5445 (S394 in *I. batatas*), F485 (F433 in *I. batatas*), N505 (N453 in *I. batatas*) and Y565 (Y515 in *I. batatas*)

Multiple sequence alignment of *I. batatas* isoprene synthase sequence together with the reference sequences is shown in FIG. 1a and FIG. 1b. Alignment of the substrate binding amino acid positions is shown in FIG. 2.

Among the additional new candidates, *Medicago sativa*, *Fragaria vesca* subsp. *Vesca*, *Morus notabilis*, *Populus trichocarpa*, *Dahlia pinnata*, *Sesamum indicum* and *Eucalyptus grandis* lacked an amino acid at F338 position (amino acid numbering based on *Populus alba* isoprene synthase), and *Mangifera indica*-derived sequence had an important amino acid at F338, which seemed to have a function of the isoprene synthase.

A multiple sequence alignment (MSA) result to an active site sequence of the isoprene synthase candidates was shown in FIG. 3. Reference isoprene synthase sequences, and four sequences (tricyclene or beta-ocimene synthase) other than the reference isoprene synthases were also included as a reference, and identity % of the sequences was shown in FIG. 5.

In FIG. 5, the reference isoprene synthases were shown in bold black text, and sequences similar to the reference isoprene synthase were shown in black text, and the candidates known in the related art were shown in black text in italics. Isoprene synthase candidates (*Ipomoea batatas* and *Elaeocarpus photiniifolius*) having 4 points of isoprene scores were shown in white text with black background. Additional new candidate sequences were shown in black text with grey background, and candidates having F388 and 3 points of isoprene score were shown in white text with dark grey background. Sequences with confirmed other functions are shown in grey. The phylogenetic tree was provided with EC number, the biological name, and Blast E-values, and was visualized by Geneious software (FIG. 4).

Example 2: Cloning of Selected Isoprene Synthase Gene Candidate

Since *Ipomoea batatas*- and *Elaeocarpus photiniifolius*-derived nucleotide sequences selected in Example 1 above have a low homology to the known isoprene synthase encoding nucleotide sequences, the polynucleotides were cloned to measure an enzyme activity of protein to be expressed by the polynucleotides.

The isoprene synthase polynucleotide candidates were expressed in *E. coli*, isoprene prepared in a culture medium was measured to confirm an activity of the isoprene synthase, and the known isoprene synthase genes (*P. alba*, *P. montana* and *A. hypogaea*-derived isoprene synthase genes) were expressed in *E. coli* as a control group. Since cloning and culturing using cyanobacteria require quite a long time to work, they were conducted by selecting *E. coli*.

The genes for cloning were synthesized while removing a portion of encoding N-terminal sequence (N-terminal 40 aa of *E. Pho* and 48aa of *I. bat*) from the gene. In all genes, codon optimization was performed on *Synechocystis* sp. PCC6803 (SEQ ID NO: 3), and then codon optimization was performed on *E. coli*, by using GeneScript.

The fabricated construct was cloned in pBAT4 (Peränen J. et al, *Anal. Biochem.*, 236:371-373, 1996) having trc promoter added thereto and cloned in pETDuet-1 (Novagen, USA, FIG. 6) having T7 promoter added thereto, so as to appropriate for expression in cyanobacteria, and when pBAT4 is used as a vector, the *P. alba*-derived polynucleotide and the *I. batatas*-derived polynucleotide were successfully cloned, and when pETDuet-1 is used as a vector, the *I. batatas*-, *E. photiniifolius*-, *P. alba*-, *P. Montana*- and *A. hypogaea*-derived isoprene synthase polynucleotides were cloned.

Example 3: Preparation of Isoprene In Recombinant Microorganism Containing Isoprene Synthase Gene Candidates

3-1: Transformation and Culturing

Plasmids containing isoprene synthase polynucleotide candidates cloned in Example 2 above were transformed into *E. coli* BL21, and expression of isoprene synthase was confirmed.

The selected transformants and parent strains were inoculated into an LB liquid medium containing 100 µg/mL ampicillin and cultured at 30° C. (or 37° C.) overnight, then the culture medium was diluted to 1:50, cultured until the OD600 at 30° C. is 0.6 to 0.7, and treated with IPTG so as to have a final concentration of 0.5 mM, thereby inducing an enzyme expression. The culturing was performed for 24 hours using a sealed 22 mL head-space bottle while inoculating the culture medium for 2 mL/bottle. At the same time, the culturing for protein analysis was performed using Erlenmeyer flask.

3-2: Detection and Quantification of Isoprene

Isoprene prepared in *E. coli* strain cloned with isoprene synthase was analyzed by solid-phase microextraction (SPME). An isoprene standard material containing 0.002% 4-tert-butylcatechol as a stabilizer (Fluka nr529240 CAS 78-79-5) was used, and analyzed using divinylbenzene/carboxen/PDMS (DVB/CAR/PDMS) fiber (2 cm). CTC Combi PAL system (CTC Analytics AG, Switzerland) was used as a sampler, and the combination of Agilent 7890A gas chromatograph (GC, Agilent Technologies, USA) with a5975C mass selective detector (MSD Agilent Technologies, USA) was used. In order to separate isoprene from ethanol which is volatile occurred during the culturing, HP-5, HP-35 (30 m) and BPX5 (60 m) columns were used, and isoprene was eluted by Lipodex (50 m) and HP-Innowax (60 m).

The elution time was 20 minutes, desorption time was 8 minutes, temperature in GC oven was between 40° C. (4 mins) and 70° C. (5° C./min), and the total running time was 16.3 mins. Temperature of the injector was 250° C., MS data were collected in m/z 35 to 350, and mass spectrum of isoprene was confirmed by comparison with NIST08 library.

The basic peak was m/z 67 and the mass peak was m/z 68, and other main fragments had m/z 53, 40 and 39. Calibration curves were measured by spike of isoprene in three different media (LB, BG11 and BG11 without Na₂CO₃). The medium had a concentration area of 2~85 ng/2 mL, and the spike was performed on the sample with ethanol having the same amount (10 µl/bottle). The sample was put into a 22 mL

head-space bottle used in both of the culturing and the analysis and was analyzed. The quantitative limit of the present invention was 0.5 ng/ml, and the detection limit was lower than the quantitative limit.

Expression in the pBAT4 constructs (trc promoter) of the *P. alba*- and *I. batatas*-derived isoprene synthase genes before and after the induction of the isoprene synthase was shown in Criterion TGX 4-20 gel with GelCode Blue dye (FIG. 7). Expression of the isoprene synthase and preparation of isoprene were confirmed by culturing five clones (A to E) of two constructs. After the induction, preparation of *P. alba*-derived isoprene synthase (64 kDa) was clearly confirmed, but *I. batatas* lysate (62 kDa) was not clearly confirmed due to high viscosity. After 24 hours of the induction, the isoprene prepared by the recombinant *E. coli* was analyzed by SPME method. As a result, it could be confirmed from FIG. 8 that the recombinant *E. coli*-derived sample containing *I. batatas*-derived isoprene synthase had particularly high isoprene productivity.

As a result of confirming expression of the isoprene synthase of the isoprene synthase clone of five kinds (*I. batatas*, *E. photiniifolius*, *P. alba*, *P. montana* and *A. hypogaea*) using pETDuet-1 vector, it could be confirmed from FIG. 9 that after 24 hours of the induction, all strains excluding *E. photiniifolius* showed clear isoprene synthase production.

As a result of analyzing the isoprene productivity in the five kinds of recombinant *E. coli*'s after 24 hours of the induction, it could be confirmed from FIG. 10 that high isoprene productivity was shown in *I. batatas*- and *P. alba*-derived isoprene synthases, in particular, the highest isoprene productivity was shown in *I. batatas*-derived isoprene synthase, and in *E. photiniifolius*, an enzyme production was not confirmed in Criterion TGX 4-20 gel, but isoprene was prepared. The preparation of isoprene was not confirmed in *A. hypogaea*.

In addition, as a result of confirming growth of each recombinant *E. coli* strain with OD₆₀₀ values, it was confirmed that the growth of each strain was slightly inhibited by expression of the recombinant enzyme (FIG. 11).

Further, FIG. 12 shows time dependent isoprene productivity after expression of isoprene synthases in an *E. coli* strain transformed into a pETDuet-1 vector in which *I. batatas* of isoprene synthases and *P. alba* of isoprene synthases, respectively, are inserted. It could be confirmed that isoprene was accumulated steadily after expression of isoprene synthases and isoprene was also produced in an *E. coli* strain without inducing expression by using IPTG

As a result of confirming time dependent growth of strain by using OD₆₀₀ value, after inducing expression by using IPTG in recombinant *E. coli* including IpS derived from *I. batatas* and recombinant *E. coli* including IpS derived from *P. alba*, growth of strain steadily decreases compared to strain without inducing treatment by using IPTG (FIG. 13).

Example 4: *Synechocystis* Sp. Cells Expressing Isoprene Synthase

Synechocystis sp. PCC6803 is used as the parent strain and is referred to as the wild type (wt). The wt strain and transformants are maintained on BG-11 agar and in BG-11 liquid medium buffered with HEPES to pH 7.5 and is supplemented with 10 mM bicarbonate and with the antibiotics kanamycin, spectinomycin or chloramphenicol as appropriate. Instead of or in addition to bicarbonate, CO₂ enriched cultivation conditions are used to provide the carbon source.

Synechocystis is cultivated at 30° C. under illumination (10-300 μmol photons m⁻²s⁻¹).

Synechocystis sp. is transformed by natural transformation using previously described methods. The presence of the transforming DNA is confirmed by PCR.

Constructs are designed for the expression of isoprene synthase in cyanobacteria. The isoprene synthase is expressed under the transcriptional control of an *E. coli* or a *Synechocystis* promoter, which allows expression of isoprene synthase in cyanobacteria. As an example, the *E. coli* trc or lac promoters or *Synechocystis* sp. psbA promoter and *Synechocystis* terminator or *E. coli* rrnB terminator or phage T7 terminator are used. The *I. batatas* isoprene synthase nucleotide sequence (SEQ ID: No. 2) was codon optimized for expression in *Synechocystis* sp. PCC6803 and synthesized by GenScript (Piscataway, N.J., USA). The expression cassettes for isoprene synthase and the antibiotic resistance marker are flanked by homologous sequences corresponding to a neutral site in the *Synechocystis* sp. genome to facilitate integration of the transforming DNA into the genome by homologous recombination.

Neutral sites e.g. between slr0168 and slr0338 in the *Synechocystis* sp. PCC6803 genome are suitable target loci for introducing the isoprene synthase. Other target sites can be chosen in order to knock out genes that potentially interfere with production of the desired product and its precursors. Genes required for biosynthesis of storage compounds such as glgA or glgC, involved in glycogen biosynthesis, are examples of such genes.

Example 5: *Synechocystis* Sp. Cells Expressing One or More Enzymes of the Mevalonate Pathway

In combination with isoprene synthase expression other genes contributing to isoprene production are introduced into *Synechocystis* cells. For example, the *idi* gene coding for isopentenyl-diphosphate delta-isomerase (EC 5.3.3.2) is introduced to enhance conversion of isopentenyl diphosphate (IPP) to dimethylallyl diphosphate (DMAPP) that is the substrate of isoprene synthase. Optionally one or more genes coding for the mevalonate pathway components, mevalonate kinase (EC2.7.1.36), phosphomevalonate kinase (EC2.7.4.2), pyrophosphomevalonate decarboxylase (EC 4.1.1.33), acetoacetyl-CoA thiolase (EC2.3.1.9), HMG-CoA synthase (EC2.3.3.10) or HMG-CoA reductase (EC1.1.1.34) are cloned under an *E. coli* or *Synechocystis* promoter that allows expression in cyanobacteria. Two or more genes are expressed as a transcriptional fusion under the control of under the transcriptional control of an *E. coli* or a *Synechocystis* promoter. Each open reading frame is preceded by a ribosome binding site (RBS) to allow independent translation of the polypeptides. The above mentioned polynucleotides and the antibiotic resistance marker are flanked by homologous sequences corresponding to the target locus of choice in the *Synechocystis* sp. genome to allow integration of the transforming construct into the genome.

Example 6: *Synechocystis* Sp. Cells Over-Expressing One or More Polypeptides of the Non-Mevalonate Pathway

Although cyanobacteria possess the non-mevalonate pathway for synthesizing isoprenoid precursors, overexpression of selected pathway components from homologous or heterologous sources will benefit isoprene production. Constructs are designed for the overexpression of the non-mevalonate pathway genes, deoxyxylulose-5-phosphate

synthase (DXP synthase; EC2.2.1.7), DXP reductoisomerase (EC1.1.1.267), MEP-cytidyltransferase (EC2.7.7.60), CPD-ME-kinase (EC2.7.1.148), MECDP synthase (1.17.7.1), HMBDP synthase (EC1.17.7.1), HMBDP reductase (EC1.17.1.2), and isopentenyl-diphosphate delta-isomerase (EC 5.3.3.2) in *Synechocystis* sp. are designed using the same principle as described for the mevalonate pathway genes. One or more non-mevalonate pathway genes are introduced into *Synechocystis* sp. in combination with isoprene synthase expression.

Example 7: Production of Isoprene by Recombinant *Synechocystis* Cells Expressing Isoprene Synthase

Synechocystis transformants expressing isoprene synthase and optional other genetic modifications are tested for isoprene production using an in vivo assay. Wild type cells are studied in parallel as controls. As an example, cells are grown for 4 days at 30° C. with 100 rpm shaking under continuous illumination in BG11 liquid medium supplemented with bicarbonate as the carbon source and the appropriate antibiotics. Cells from 20 ml cultivation are harvested by centrifugation and suspended in 8 ml of BG11 medium supplemented with bicarbonate and antibiotics as appropriate in 20 ml GC-MS bottles which are sealed air-tight. When isoprene synthase is expressed under control of an IPTG inducible promoter 0.5 mM IPTG is added into medium to induce isoprene production. Cultivations are also carried out without IPTG. The bottles are incubated at 30° C. with 100 rpm shaking for up to 240 h. Samples are taken at regular intervals during the cultivation. At each time point a bottle is removed, the cultivation is heated at 40° C. and isoprene is measured from the head space of the bottle using the GC-MS method described earlier for *E. coli*.

Isoprene production into the medium in aerobic cultures of *Synechocystis* is also analysed. The culture medium is sampled periodically and the samples are transferred into GC-MS vials, heated at 40° C., and isoprene is measured off-line from the head space of the bottle using the GC-MS method described earlier for *E. coli*.

Example 8: Production of Isoprene by Recombinant *Synechocystis* Cells in a Bioreactor

The inocula for bioreactor cultivation are grown at 30° C. with 100 rpm shaking under continuous illumination in BG11 liquid medium supplemented with bicarbonate as the carbon source and the appropriate antibiotics. The inoculum is transferred into BG11 medium in a photobioreactor. Bicarbonate or CO₂ gas is used as the carbon source. The photobioreactor is equipped with adjustable lighting and controllable gas inlet and outlet. The cells are grown at 30° C. and the cultivation is mixed with agitation or gas bubbling. Isoprene production is analysed off-line. The culture medium is sampled periodically and the samples are transferred into GC-MS vials, heated at 40° C., and isoprene is measured from the head space of the bottle using the GC-MS method described earlier for *E. coli*. Isoprene is harvested from the bioreactor flushing the reactor periodically with CO₂ as described (Bentley and Melis, 2012, Biotechnol Bioeng 109:100-109).

Example 9: Construction of Isoprene Producing *Saccharomyces Cerevisiae* Strains

Constructs are designed for the expression of isoprene synthase in *S. cerevisiae*. The isoprene synthase nucleotide

sequence is expressed from an autonomously replicating multi-copy vector containing a 2-micron replication origin or an ARS sequence, a centromeric vector, or integrated into the genome in one or more copies. Optional other genetic modifications such as overexpression of isopentenyl-diphosphate delta-isomerase (IDI) or mevalonate pathway or non-mevalonate pathway components are also introduced into *S. cerevisiae*. The constructs can be assembled by traditional cloning methods or by recombination cloning. For example, *S. cerevisiae* strain FY834 is used for recombination cloning (Winston, Dollard and Ricupero-Hovasse, 1995). *S. cerevisiae* strain H2798 (ura-) is the parental strain which is used for the production of ispS isoprene.

A vector, named SP17, containing three *S. cerevisiae* promoter-terminator pairs cloned into the pRS426 plasmid, is used. The truncated Hmg-CoA reductase (Polakowski et al. 1998, Appl Microbiol Biotechnol. 49:66-71) is amplified by PCR using the oligonucleotides TAGCAATCTAATCTAAGTTTAAATTA-CAAACTCGAGTAAAAATGGACCAA TTTGGT-GAAAACCTG (SEQ ID NO: 4) and CCAAACCTCTGGCGAAGAAGT CCAAAGCTGTCGACGGATTTAATGCAGGTGACGG (SEQ ID NO: 5) and cloned into a SP17 vector between e.g. the TEF1 promoter and ADH1 terminator by homologous recombination. The IspS nucleotide sequence of *I. batatas* (SEQ ID NO: 2) is codon optimized for expression in *S. cerevisiae* and synthesized by GenScript, amplified by PCR, and inserted between e.g. the PGK1 promoter and PGK1 terminator. The IDI nucleotide sequence is amplified by PCR and cloned between e.g. TPI1 promoter and CYC1 terminator by homologous recombination. Schematic representation of the construct is shown in FIG. 14. *S. cerevisiae* are transformed using the LiAc/PEG method (Gietz and Woods, 2002, Methods in Enzymology 350: 87-96). The resulting plasmid is transformed into *S. cerevisiae* strain and selected on synthetic complete medium lacking uracil (SCD-Ura). The presence of the transforming DNA is confirmed by PCR.

The *S. cerevisiae* IspS transformants are propagated aerobically at 30° C. with 250 rpm shaking in SC-Ura medium containing glucose, galactose or glycerol as the carbon source to produce the biomass for isoprene production. For isoprene production, aliquots of the precultures are transferred into fresh SC-Ura medium containing glucose, galactose or glycerol in tightly sealed flasks, which enable harvesting of isoprene. The bottles are incubated at 30° C. with 250 rpm shaking for up to 96 h. Samples are taken at regular intervals during the cultivation. At each time point a bottle is removed, the cultivation is heated at 40° C. and isoprene is measured from the head space of the bottle using the GC-MS method described earlier for *E. coli*.

Example 10: Construction of *Pichia kudriavzevii* Strains for Isoprene Production

Synthetic *I. batatas* IspS gene, optimized for expression in *S. cerevisiae*, was obtained from GenScript (Piscataway, N.J., USA). The IspS polynucleotide was introduced into *P. kudriavzevii* strain ATCC32196 under the PGK1 or TDH1 promoters of *P. kudriavzevii* together with the isopentenyl-diphosphate delta-isomerase (IDI) gene. The *P. kudriavzevii* PGK1 promoter and *S. cerevisiae* MEL5 terminator controlled the expression of the hygromycin resistance marker that was used for selection of transformants. The Pk promoters were amplified from the genomic DNA of *P. kudriavzevii* strain ATCC32196 essentially as described in US

2009/0226989 A1. Terminators from *S. cerevisiae* and *P. kudriavzevii* were used. The marker cassette was flanked by loxP sites to enable removal and re-use of the marker. The isoprene synthase and marker expression cassettes were flanked by homologous sequences corresponding to the *P. kudriavzevii* GPD1 locus to enable integration in the GPD1 locus. The resulting construct contained P.k. GPD1 5' flanking region-P.k. PGK1 promoter-MEL5 or hygromycin marker-MEL5 terminator-P.k. promoter-isoprene synthase nucleotide sequence-P.k. or S.c. terminator-P.k. promoter-IDI-P.k. or S.c.-P.k. GPD1 3' flanking region (FIG. 15).

A HMG-CoA reductase (Polakowski et al. 1998, Appl Microbiol Biotechnol. 49:66-71) was co-expressed with the isoprene synthase in *P. kudriavzevii* under control of the *P. kudriavzevii* promoter. The resulting construct contained P.k. PDC1 3' flanking region-P.k. promoter-MEL5 or hygromycin marker-MEL5 terminator-P.k. promoter-HMG-CoA reductase nucleotide sequence-P.k. terminator-P.k. PDC1 5' flanking region (FIG. 16).

The resulting constructs were transformed into *P. kudriavzevii* strain ATCC32196 using the LiAc/PEG method. The transformants were selected based on blue colour on yeast peptone dextrose (YPD) medium containing 5-bromo-4-chloro-3-indolyl- α -D-galactopyranoside (X-alpha-gal) or for growth on melibiose, or for growth on YPD medium containing hygromycin essentially as described in US 2009/0226989 A1. The presence of the transforming DNA was confirmed by PCR. Transformants were cultured aerobically on synthetic complete medium or yeast peptone (YP) medium containing glucose, fructose or glycerol as the carbon source. For isoprene analysis, aliquots of the precultures are transferred into fresh medium in tightly sealed GC-MS bottles retaining isoprene. The bottles were incubated at 30° C. with 100 rpm shaking for up to 96 h. Samples were taken at regular intervals during the cultivation. At each time point a bottle was removed, the cultivation was heated at 40° C. and isoprene was measured from the head space of the bottle using the GC-MS method described earlier for *E. coli* except that the medium used for the calibration curve was YP-medium containing 0.5% ethanol.

The expression vector for an N-terminally truncated *S. cerevisiae* HMG1 was named pPK-HMG (FIG. 16, SEQ ID NO: 6) and targeted for integration into the *P. kudriavzevii* PDC1 locus. The N-terminally truncated HMG1 gene (SEQ ID NO: 34) was amplified from genomic DNA of *S. cerevisiae* W303 by PCR using primers (5'-AGCGCGGATCCATGGACCAATTGGTGAAGAACTGAAG) (SEQ ID NO: 7) and (5'-AGCGCGGATCCCA-CATGGTGCTGTTGTGCTTC) (SEQ ID NO: 8) and expressed under the control of the *P. kudriavzevii* PGK1 promoter and *S. cerevisiae* ADH1 terminator. The expression construct with the MEL5 marker and *P. kudriavzevii* PDC1 homology regions was released with NotI from plasmid pPK-HMG and transformed into *P. kudriavzevii* VTT-C-79090T (ATCC32196) using the lithium acetate method and colonies were screened based on blue colour on YPD-agar containing 40 μ g/ml 5-bromo-4-chloro-3-indolyl- α -D-galactopyranoside. A transformant Pk/HMG1 was selected for continuation.

The vector for expression of *I. batatas* IspS and *S. cerevisiae* IDI1 was named pPK-IspS-IDI (FIG. 15; SEQ ID NO: 9). The integration was targeted to the *P. kudriavzevii* GPD1 locus. The *P. kudriavzevii* GPD1 homology regions were amplified by PCR from genomic DNA of *P. kudriavzevii* VTT-C-79090T (ATCC32196). The *I. batatas* IspS gene with a C-terminal StrepII-tag was codon optimized for expression in *S. cerevisiae* and synthesized by Genscript

(Hongkong) (SEQ ID NO: 33). *I. batatas* IspS was expressed under control of the *P. kudriavzevii* PGK1 promoter and *S. cerevisiae* ADH1 terminator. The IDI1 gene (SEQ ID NO: 35) was amplified by PCR from genomic *S. cerevisiae* DNA using primers 918IDISc-F (5'-CTTTTCAACAACAATATAAAAACCAAAAAGCGGCCGCTTAAT-TAAAAAATGACTGCCGACAACAATAGTATG) (SEQ ID NO: 10) and C031_919new_IDISc_R (5'-AGAGACATGGGAGATCCCGCGGGCGGCCGCTTAATTAAT-TATAGCATTCTATGAATTTGCC) (SEQ ID NO:11), and placed under the control of the *P. kudriavzevii* TDH1 promoter and *S. cerevisiae* PGK1 terminator. The expression construct with a hygromycin resistance marker and *P. kudriavzevii* GPD1 homology regions was released with NotI from plasmid pPK-IspS-IDI and transformed into *P. kudriavzevii* VTT-C-79090T (ATCC32196) and into *P. kudriavzevii* Pk/HMG1 using the lithium acetate method (Gietz et al. 1992, Nucleic Acids Res 20:1425-1425) and colonies were selected on YPD-agar containing 450 μ g/ml hygromycin B as the selective agent. Transformed colonies Pk/IspS+IDI1-69, Pk/IspS+IDI1-72, Pk/IspS+IDI1-74 and Pk/IspS+IDI1+HMG1-79 were isolated and tested for isoprene production.

P. kudriavzevii VTT-C-79090T (ATCC32196) and the Pk/HMG1, Pk/IspS+IDI1 and Pk/IspS+IDI1+HMG1 transformants were grown in YPD medium o/n at 30° C. Cells were collected by centrifugation and suspended in YP+1% glucose or YP+1% glycerol to an OD₆₀₀=3.2 ml aliquots were sealed in 22 ml headspace bottles and incubated o/n at 30 with 100 rpm shaking. Isoprene production was measured as described above except that the isoprene standards were prepared in YP-medium containing 0.5% ethanol. Isoprene concentrations measured are shown in FIG. 18. FIG. 18 shows isoprene production by *P. kudriavzevii* transformants in YP medium containing 1% glucose (grey) or 1% glycerol (black) as the carbon source. All transformants expressing IspS and IDI1 produced isoprene while the parent strain and the strain expressing only HMG1 did not produce detectable isoprene. The amount of isoprene measured ranged from 1 to 12 μ g in the 2 ml samples depending on the strain and cultivation conditions. More isoprene was produced on YP-glycerol medium than on YP-glucose medium. The presence of HMG1 in addition to IspS and IDI1 increased isoprene production approximately 3-fold in YP-glycerol medium and 2-fold in YP-glucose medium.

Example 11: Construction of *Trichoderma Reesei* Strains Overexpressing an Isoprene Synthase for Isoprene Production I

Synthetic *I. batatas* IspS gene, optimized for expression in *T. reesei*, is obtained from GenScript (Piscataway, N.J., USA) (SEQ ID NO: 36). The codon optimized IspS polynucleotide was introduced into *T. reesei* strain Rut-30 under the control of *gpdA* promoter of *Aspergillus nidulans* or under the control of the *cbh1* promoter of *T. reesei*.

The resulting constructs contained the *T. reesei* *cbh1* 5' flank region-*cbh1* promoter—isoprene synthase—nucleotide sequence *A. nidulans* *gpdA* promoter hygromycin resistance marker (*hph*) and *A. nidulans* *trpC* terminator—*T. reesei* *cbh1* terminator and 3' flank region. The resulting constructs were transformed into *T. reesei* using the PEG-mediated protoplast transformation. The transformants were selected based on hygromycin resistance. Instead of *hph*, the acetamidase *A. nidulans* *amdS* gene can be used as the selective marker and the transformants were selected based on the ability to use acetamide as the sole nitrogen source.

Alternatively, the isoprene synthase was expressed under the *A. nidulans* *gpdA* promoter. The codon optimized isoprene synthase polynucleotide was cloned into an expression vector containing the *A. nidulans* *gpdA* promoter—*isoprene synthase*—nucleotide sequence—*A. nidulans* *trpC* terminator—*A. nidulans* *gpdA* promoter hygromycin resistance marker (*hph*) and *A. nidulans* *trpC* terminator. Schematic view of the plasmid used for transformation was shown in FIG. 16. The transformants were purified from colonies originating from single spores, and the integration of the expression cassette into the genome was confirmed by PCR amplification of the expression cassette. The polynucleotides were integrated into the *cbh1* locus or into a non-homologous site in *T. reesei* genome.

T. reesei transformants containing the *ispS* polynucleotide were cultivated in shake flasks in medium containing 7.6 g/L $(\text{NH}_4)_2\text{SO}_4$, 15.0 g/L KH_2PO_4 , 2.4 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 4.1 mM $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, 3.7 mg/L CoCl_2 , 5 mg/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1.4 mg/L $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1.6 mg/L $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$, and a 10 g/L carbon source or a mixture of carbon sources selected from cellulose, xylan, glucose, lactose, glycerol, galactose, sorbose, sorbitol, xylose, arabinose or 0.7 mM sophorose. The pH of the medium was adjusted to 4.8 by addition of KOH. The cultures were inoculated with 8×10^7 spores/200 ml medium and grown for up to 7 days in conical flasks at 28° C. with shaking at 250 rpm. The culture medium was sampled periodically and the samples were transferred into GC-MS vials, heated at 40° C., and isoprene was measured off-line from the head space of the bottle using the GC-MS method described earlier for *E. coli*.

Alternatively, the aliquots of the culture transferred into fresh medium in tightly sealed bottles. The bottles were incubated at 28° C. with 250 rpm shaking for up to 96 h. Samples were taken at regular intervals during the cultivation. At each time point a bottle was removed, the cultivation was heated at 40° C. and isoprene was measured from the head space of the bottle using the GC-MS method described earlier for *E. coli*.

Example 12: Construction of *Trichoderma Reesei* Strains Overexpressing an Isoprene Synthase for Isoprene Production II

Strains

Escherichia coli DH5 α (Life Technologies) was used for propagation of the plasmids. *Trichoderma reesei* QM6a (VTT-D-071262T, ATCC13631), RutC-30 (VTT-D-086271, ATCC56765), and M122 (RutC-30 mus53A) were obtained from VTT Culture Collection (Espoo, Finland). Spore suspensions were prepared by cultivating the fungus on potato-dextrose plates (BD, Sparks, Md., USA) for 5-7 days, after which the spores were harvested, suspended in a buffer containing 0.8% NaCl, 0.025% Tween-20 and 20% glycerol, filtered through cotton and stored at -80° C.

Expression Vector

The vector for expression of *I. batatas* *IspS* in *T. reesei* was named pCIL-105 (FIG. 19 SEQ ID NO:12). The backbone for the expression vector contained 5' and 3' flank regions (<1000 bp) of the *T. reesei* *pep4* (*tre77579*) gene in pRS426 (ATCC77107) vector background. The primer sequences indicating the start and end regions for both *pep4* flanks are shown in Table 1. The selection marker (*pyr4*) in the vector backbone pCIL102 was removed by NotI digestion and replaced by hygromycin resistance cassette obtained from plasmid pCIL-107 by NotI digestion. The gene encoding *I. batatas* isoprene synthase with a C-terminal StrepII-tag was obtained from *E. coli* expression plasmid pCIL41 by NdeI-AvrII digestion. The *Aspergillus nidulans* *gpdA* promoter, *A. nidulans* *trpC* terminator and a bridge fragment (part of *pep4* 3' flank) were produced by PCR using primers in Table 1 and plasmid pCIL104 containing these elements as templates. PCR amplification was carried out with KAPA HiFi HotStart ReadyMix PCR kit (KAPA Biosystems). All fragments were separated with agarose gel electrophoresis and isolated with a gel extraction kit (Qiagen). The PCR fragments contained 30 bp or 40 bp overlapping sequences needed for cloning the expression construct using homologous recombination system in yeast. The vector backbone and the appropriate digestion and PCR fragments were transformed into *S. cerevisiae* (strain H3488/FY834) according to Gietz, R. D., and Woods, R. A. (2002, Guide to Yeast Genetics and Molecular and Cell Biology, Pt B 350, pp. 87-96). The plasmid DNA from the yeast transformants was rescued by transformation into *E. coli* and checked from a few clones by digestion. The clone taken further was verified by sequencing and named pCIL105 (SEQ ID NO: 12).

Strain Generation

For *T. reesei* transformation, the expression construct was released from pCIL105 with MspI and the correct fragment was purified from agarose gel using QIAquick Gel Extraction Kit (Qiagen). *T. reesei* strain RutC-30 or *T. reesei* strain M122 (RutC-30 Amus53) was transformed with 5 μg of expression cassette fragment according to Penttilä et al. 1987 (Gene 61: 155-164). Transformants were selected on *Trichoderma* minimal medium containing 125 $\mu\text{g}/\text{ml}$ hygromycin B (Calbiochem). The transformants were streaked onto *Trichoderma* minimal medium agar with 0.1% (v/v) Triton X-100 and 150 $\mu\text{g}/\text{ml}$ Hygromycin B and cultivated at +28° C. Transformants growing on selective plates were screened by PCR for correct 5' and 3' integration or for the presence of the *IspS* expression cassette (FIG. 20) somewhere else in the genome using the primers shown in Table 2. FIG. 20. shows PCR analysis for the presence of the *IspS* expression cassette in *T. reesei* transformed with pCIL105. Wild type strain QM6a, parent and DDIW are negative controls. For PCR, internal primers (SEQ ID NO: 31 and SEQ ID: NO 32) shown in Table 2 were used. The expected product size was approximately 1.1 kb.

TABLE 1

Primers used to generate fragments for cloning the expression vector pCIL105 for <i>I. batatas</i> <i>IspS</i> -C-StrepII.		
Primer	Sequence	Product
77579_5f	TCAGGTCAACCACCGAGGAC (SEQ ID NO: 13)	<i>pep4</i> 5' flank
77579_5r	TGAATGGGATGGTTCGATTG (SEQ ID NO: 14)	
77579_3f	AGGTAGACGCTTTGCGAGTG (SEQ ID NO: 15)	<i>pep4</i> 3' flank
77579_3r	TGAACTGACGCGGACTGA (SEQ ID NO: 16)	

TABLE 1-continued

Primers used to generate fragments for cloning the expression vector pCIL105 for <i>I. batatas</i> IspS-C-StrepII.		
Primer	Sequence	Product
C017_gpdA_rec_for	CCTCTGGCAGCAATCGAACCATCCCATTTCATTAATTAA GCTCCTTATTGAAGTCGGAGG (SEQ ID NO: 17)	gdpA prom
C018_gpdA_rec_rev	ATGAGGAGGGTTGATAGTTTGTCTGAGCGGGCGGCGAGT CATGATGTCTGCTCAAGCGGG (SEQ ID NO: 18)	
C019_trpC_rec_for	TAATCCAGTGGGAATGGTCCCACCCACAATTTGAAAAG TAAGATCCACTTAACGTTACTGAAATCA (SEQ ID NO: 19)	trpC term
C020_trpC_rec_rev	AAGGGGACCGGCCGCTAGTCTCACCGTTATGCGGCCG CTTAATTAAGAGTGGAGATGTGGAGTGGG (SEQ ID NO: 20)	
C021_pep4_3frec_for	GATAACCCATCGGCAGCAGATGATAATGATTCCGCAG CACGCGGCCGAGGTAGACGCTT (SEQ ID NO: 21)	pep4 bridge
C022_pep4_3f rev	GAGGTGCCAAAGCCGTTTCG (SEQ ID NO: 22)	

TABLE 2

Primers used to screen for correct integration of the expression cassette or the presence of the IspS cassette in the <i>T. reesei</i> genome.		
Primer	Sequence	Product
T302_77579_5int	GATTCATCACAGGGGCGAGTC (SEQ ID NO: 23)	5'integration
T624_gpdA_seqR1	CTCCATATTCTCCGATGATGC (SEQ ID NO: 24)	
T302_77579_5int	GATTCATCACAGGGGCGAGTC (SEQ ID NO: 25)	5'integration
C046_gpdA_rev	TATCCTCTTGACACCGCTCC (SEQ ID NO: 26)	
T415_77579_3screen	ACGCCGTTGCTGAGCCTTG (SEQ ID NO: 27)	3'integration
T1411_cbh2t_end_f	CCAATAGCCCGGTGATAGTC (SEQ ID NO: 28)	
T415_77579_3screen	ACGCCGTTGCTGAGCCTTG (SEQ ID NO: 29)	3'integration
T1404_cbh2term_for	CCGCTAGCGCTGTTGATTG (SEQ ID NO: 30)	
C009_ibat_for1	GATCAACTTAGCACGCATTG (SEQ ID NO: 31)	internal
C029_PKIp_rev	TTTGCTCCAACCTCAGGCG (SEQ ID NO: 32)	

Example 13: Construction of *Aspergillus Niger* Strains Overexpressing an Isoprene Synthase for Isoprene Production

The codon optimized isoprene synthase polynucleotide of example 3 was cloned into an expression vector containing the *A. nidulans* gpdA promoter—*isoprene synthase* polynucleotide—*A. nidulans* trpC terminator—*A. nidulans* gpdA promoter hygromycin resistance marker (hph) and *A. nidulans* trpC terminator (FIG. 17). The resulting construct was transformed into *A. niger*. Transformation was performed using the PEG-mediated protoplast transformation. The transformants were selected based on hygromycin resistance. The presence of the transforming DNA was confirmed by PCR.

A. niger was grown in the production medium (The defined medium of Vogel described by Mojzita et al., 2010, with 20 g/L glucose or xylose as a carbon source). Pre-cultures were grown in the medium containing 10 g/L yeast extract, 20 g/L peptone and 30 g/L gelatine (50 ml medium in 250 ml flasks). Mycelium from 50 ml cultures was collected by filtration, washed with sterile H₂O and re-suspended in 20 ml of the production medium in 250 ml flasks. Cultures were incubated at 30° C., 250 rpm. The

culture medium was sampled periodically and the samples were transferred into GC-MS vials, heated at 40° C., and isoprene was measured off-line from the head space of the bottle using the GC-MS method described earlier for *E. coli*. Alternatively, for isoprene production, aliquots of the culture were transferred into fresh medium in tightly sealed GC-MS bottles. The bottles were incubated at 30° C. with 250 rpm shaking for up to 96 h. Samples were taken at regular intervals during the cultivation. At each time point a bottle was removed, the cultivation was heated at 40° C. and isoprene was measured from the head space of the bottle using the GC-MS method described earlier for *E. coli*.

Example 14: *Ipomoea Batatas* IspS Expression in Plant Cell Cultures

The *I. batatas* isoprene synthase polynucleotide was cloned into an over expression vector functional in various plant species under the control of the CaMV35S promoter for expression in plant cells. The expression vector had the hygromycin or kanamycin resistance marker for the selection of transformants. *Nicotiana tabacum* bright yellow (BY-2) cell suspension cultures or hairy root cultures were maintained and transformed with the isoprene synthase

expression construct essentially as described in (Häkkinen et al. 2007 *Phytochemistry* 68:2773-2785). Transformants were selected based on antibiotic resistance. The presence of the transforming DNA was confirmed by PCR. For isoprene accumulation analysis, hairy roots were incubated in 20 ml medium in 100 ml shake flasks and cultivated in a rotary shaker (70 rpm, 24° C.) in modified Gamborg B5 medium without casein (Jouhikainen et al., *Planta*, 208:545-551, 1999) for up to 28 days. The culture medium was sampled periodically and the samples were transferred into GC-MS

vials, heated at 40° C., and isoprene was measured off-line from the head space of the bottle using the GC-MS method described earlier for *E. coli*.

Although specific embodiments of the present invention are described in detail, it will be apparent to those skilled in the art that the specific description is merely desirable exemplary embodiment and should not be construed as limiting the scope of the present invention. Therefore, the substantial scope of the present invention is defined by the accompanying claims and equivalent thereof.

SEQUENCE LISTING

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50          55          60
Glu Tyr Leu Val Asp Thr Thr Thr Asn Asp Ser Lys Leu Arg Ile Gln
65          70          75          80
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85          90          95
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165         170         175
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Lys Trp Trp Lys Asp Thr Ala Leu Ala Asp Lys Leu Ser Phe Ala Arg
290         295         300

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 340 345 350

Leu Glu Lys Phe Thr Ala Ala Ala Glu Arg Trp Asp Val Asp Ala Ile
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Lys Asp Leu Pro Asp Tyr Met Lys Leu Cys Tyr Leu Ser Leu Phe Asn
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Val Ile Pro Ile Met Lys Lys Ala Trp Ala Asp Leu Leu Lys Ala Phe
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Leu Gln Glu Ala Gln Trp Ile Tyr Asn Lys Tyr Thr Pro Thr Phe Asp
 420 425 430

Glu Tyr Leu Asn Asn Ala Arg Phe Ser Val Ser Gly Cys Val Met Leu
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Val His Ser Tyr Phe Thr Thr Gln Asn Ile Thr Lys Glu Ala Ile His
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Ser Leu Glu Asn Tyr His Asp Leu Leu Ile Trp Pro Ser Ile Val Phe
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<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: optimized sequence of IspS

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<213> ORGANISM: Artificial
<220> FEATURE:
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<400> SEQUENCE: 4

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<210> SEQ ID NO 5
<211> LENGTH: 53
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer

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<400> SEQUENCE: 5

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<210> SEQ ID NO 6
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<212> TYPE: DNA
<213> ORGANISM: Artificial
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<223> OTHER INFORMATION: expression vector for an N-terminally truncated
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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1189)..(1189)
<223> OTHER INFORMATION: n is a, c, g, or t
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<223> OTHER INFORMATION: n is a, c, g, or t

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<400> SEQUENCE: 6

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36

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<400> SEQUENCE: 8

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 <223> OTHER INFORMATION: n is a, c, g, or t

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<220> FEATURE:
<223> OTHER INFORMATION: C018_gpdA_rec_rev primer

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<400> SEQUENCE: 18
atgaggaggg ttgatagttt gctgagcggc gggcagtcac gatgtctgct caagcggg 58

<210> SEQ ID NO 19
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: C019_trpC_rec_for primer

<400> SEQUENCE: 19
taatccagtg gaatggtccc acccacaatt tgaaaagtaa gatccactta acgttactga 60
aatca 65

<210> SEQ ID NO 20
<211> LENGTH: 66
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: C020_trpC_rec_rev primer

<400> SEQUENCE: 20
aaggggaccg gccgctagtc tcaccgttat gcggccgctt aattaagagt ggagatgtgg 60
agtggg 66

<210> SEQ ID NO 21
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: C021_pep4_3frec_for primer

<400> SEQUENCE: 21
gataacccat cggcagcaga tgataatgat tccgcagcac gcggccgcag gtagacgctt 60

<210> SEQ ID NO 22
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: C022_pep4_3f_rev primer

<400> SEQUENCE: 22
gaggtgccaa agccggttctg 20

<210> SEQ ID NO 23
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: T302_77579_5int primer

<400> SEQUENCE: 23
gattcatcac aggggcagtc 20

<210> SEQ ID NO 24
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: T624_gpdA_seqR1 primer

<400> SEQUENCE: 24

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ctccatattc tccgatgatg c	21
<210> SEQ ID NO 25 <211> LENGTH: 20 <212> TYPE: DNA <213> ORGANISM: Artificial <220> FEATURE: <223> OTHER INFORMATION: T302_77579_5int primer <400> SEQUENCE: 25	
gattcatcac aggggcagtc	20
<210> SEQ ID NO 26 <211> LENGTH: 20 <212> TYPE: DNA <213> ORGANISM: Artificial <220> FEATURE: <223> OTHER INFORMATION: C046_gpdA_rev primer <400> SEQUENCE: 26	
tatcctcttg acaccgctcc	20
<210> SEQ ID NO 27 <211> LENGTH: 19 <212> TYPE: DNA <213> ORGANISM: Artificial <220> FEATURE: <223> OTHER INFORMATION: T415_77579_3screen primer <400> SEQUENCE: 27	
acgccggtgc tgagccttg	19
<210> SEQ ID NO 28 <211> LENGTH: 20 <212> TYPE: DNA <213> ORGANISM: Artificial <220> FEATURE: <223> OTHER INFORMATION: T1411_cbh2t_end_f primer <400> SEQUENCE: 28	
ccaatagccc ggtgatagtc	20
<210> SEQ ID NO 29 <211> LENGTH: 19 <212> TYPE: DNA <213> ORGANISM: Artificial <220> FEATURE: <223> OTHER INFORMATION: T415_77579_3screen primer <400> SEQUENCE: 29	
acgccggtgc tgagccttg	19
<210> SEQ ID NO 30 <211> LENGTH: 20 <212> TYPE: DNA <213> ORGANISM: Artificial <220> FEATURE: <223> OTHER INFORMATION: T1404_cbh2term_for primer <400> SEQUENCE: 30	
ccgtctagcg ctgttgattg	20
<210> SEQ ID NO 31 <211> LENGTH: 20 <212> TYPE: DNA <213> ORGANISM: Artificial	

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<220> FEATURE:
 <223> OTHER INFORMATION: C009_Ibat_for1 primer

<400> SEQUENCE: 31

gatcaactta gcacgcattg 20

<210> SEQ ID NO 32
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: C029_PKIp_rev primer

<400> SEQUENCE: 32

tttgctccaa ctcaggcg 18

<210> SEQ ID NO 33
 <211> LENGTH: 1650
 <212> TYPE: DNA
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: polynucleotide sequence encoding the isoprene synthase optimized by codon optimization for yeast

<400> SEQUENCE: 33

atgacagcca gaagatcagc aaactatcaa ccttcacatc ggtcctacga cgaatacttg 60
 gtcgatacaa caacaaacga ttctaaatta agaatacaag aagacgcaag aaagaaattg 120
 gaagaagaag taagaaacgt tttggaagat ggtaaattag aaactttggc cttgttgga 180
 ttgatcgatg acattcaaag attgggttta ggttacaagt ttagagaatc aacatccacc 240
 agtttagcca tgttgaagat gtcagttggt caagaagcat ctaattcttc tttgcattct 300
 tgttcattgt actttagatt gttgagagaa cacggtttcg atataacacc agacgtattc 360
 gaaaaattca aggatgaaaa cggtaaattc aaagattcta tcgctaagga tgtagaggt 420
 ttgttaagtt tatatgaagc atcatttttg ggtttcgaag gtgaaaacat attggatgaa 480
 gctagagagt ttactacaat gcacttgaat aacatcaagg ataaggtaaa cccaagaata 540
 gcagaagaag taaacatgac cttggaatta cctttgcaca gaagagttga aagattagaa 600
 gctagaagaa gaatacaatc ctacagtaag tctggtgaaa ccaatcaagc attggtgact 660
 ttggcaaaga tcgatttcaa cactgtocaa gcagtatacc aaagagattt gcaagacggt 720
 tcaaaatggt ggaaggacac agctttagca gataaattgt ccttcgcaag agatagattg 780
 atggaatctt tcttttgggc catcgcatg tcttacgac cacaacactc aaagtccaga 840
 gaagctgtca ctaagacttt taaattggtt accgtcttgg atgacgttta tgacgtctac 900
 ggttctttag atgaattgga aaaattcact gctgcagccg aaagatggga tgtagacgct 960
 ataaaagatt tgctgacta catgaagttg tgttacttat cattgtttaa tactgttaac 1020
 gatttggcat atgacacatt gaaagataag ggtgaaaccg tcattccaat aatgaaaaag 1080
 gcttgggctg atttgttaaa agccttctta caagaagctc aatggatcta taataagtac 1140
 acccctactt tcgatgaata cttaaataac gctagattca gtgtttctgg ttgogtaatg 1200
 ttggttcatt cttactttac cactcaaac atcacaaagg aagcaatcca tagtttgga 1260
 aactaccacg acttgtaaat atggccttct atcgtcttca gattagctaa tgattgtcc 1320
 agttctaagg cagaatcga aagagtgaa actgccaatt ctatccatg ttacatgaa 1380
 gaaacaggtc aatcagaaga acaagctaga gaacatatct ccaaattgat cgatgaatgc 1440
 ttcaaaaaga tgaataagga aatggtggcc acatcaacct cccatttga aaaatcattc 1500

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atcgaacccg ctattaactt agcaagaatt gccttgtgcc aatatcaata cggtagcgc 1560
cactctgac ctgacgtag agcaagaaat agaatcgtaa gtgtcatcat taatccagtc 1620
gaatggtcac atccacaatt tgaagaagtaa 1650

```

```

<210> SEQ ID NO 34
<211> LENGTH: 1578
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Truncated S. cerevisiae HMGI ORF

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```

<400> SEQUENCE: 34
atggaccaat tggtgaaaac tgaagtcacc aagaagtctt ttactgctcc tgtacaaaag 60
gcttctacac cagttttaac caataaaaca gtcatttctg gatcgaaagt caaaagttta 120
tcatctgcgc aatcgagctc atcaggacct tcatcatcta gtgaggaaga tgattccgc 180
gatattgaaa gcttggataa gaaaatacgt cctttagaag aattagaagc attattaagt 240
agtggaaata caaaacaatt gaagaacaaa gaggtcgctg ccttggttat tcacggtaag 300
ttacctttgt acgctttgga gaaaaaata ggtgatacta cgagagcggg tgcggtacgt 360
aggaaggctc tttcaatfff ggacagaagct cctgtattag catctgatcg tttaccatat 420
aaaaattatg actacgaccg cgtatttggc gcttgtgtg aaaatgttat aggttacatg 480
cctttgcccg ttggtgttat aggcccttg gttatcgatg gtacatctta tcatatacca 540
atggcaacta cagagggttg tttgtagct tctgccatgc gtggctgtaa ggcaatcaat 600
gctggcggtg gtgcaacaac tgttttaact aaggatggta tgacaagagg cccagtagtc 660
cgtttcccaa ctttgaagag atctggtgcc tgtaagatat ggtagactc agaagaggga 720
caaaacgcaa ttaaaaaagc ttttaactct acatcaagat ttgcacgtct gcaacatatt 780
caaaactgtc tagcaggaga tttactcttc atgagattta gaacaactac tggtagcgc 840
atgggtatga atatgatttc taaagggtgc gaatactcat taaagcaaat ggtagaagag 900
tatggctggg aagatatgga ggttgtctcc gtttctgta actactgtac cgacaaaaaa 960
ccagctgcc acaactggat cgaaggtcgt ggtaagagtg tcgtcgcaga agctactatt 1020
cctggtgatg ttgtcagaaa agtggttaaaa agtgatgttt ccgcattggt tgagttgaac 1080
attgctaaga atttggttg atctgcaatg gctgggtctg ttggtggatt taacgcacat 1140
gcagctaatt tagtgacagc tgtttctctg gcattaggac aagatcctgc acaaaatggt 1200
gaaagttcca actgtataac attgatgaaa gaagtggacg gtgatattgag aatttccgta 1260
tccatgccat ccacggaagt aggtaccatc ggtggtgta ctgttctaga accacaaggt 1320
gccatggttg acttattagg tgaagagggc ccgcatgcta ccgctcctgg taccacgcga 1380
cgtcaattag caagaatagt tcctgtgcc gtcttggcag gtgaattatc cttatgtgct 1440
gccctagcag ccggccatft ggttcaaaat catatgacct acaacaggaa acctgctgaa 1500
ccaacaaaac ctaacaatft ggacgccact gatataaatc gtttgaaga tgggtccgct 1560
acctgcatta aatcctaa 1578

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<210> SEQ ID NO 35
<211> LENGTH: 867
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: S. cerevisiae IDI1 ORF

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<400> SEQUENCE: 35

```

atgactgccc acaacaatag tatgccccat ggtgcagtat ctagttagcg caaattagtg    60
caaaacccaa cacctgaaga cttttggaa gagtttcctg aaattattcc attacaacaa    120
agacctaaata cccgatctag tgagacgtca aatgacgaaa gcggagaaac atgtttttct    180
ggtcatgatg aggagcaaat taagttaatg aatgaaaatt gtattgtttt ggattgggac    240
gataatgcta ttggtgcccg taccaagaaa gtttgcatt taatggaaaa tattgaaaag    300
ggtttactac atcgtgcatt ctccgtcttt attttcaatg aacaagggtg attactttta    360
caacaaagag ccaactgaaa aataactttc cctgatcttt ggactaacac atgctgctct    420
catccactat gtattgatga cgaattaggt ttgaagggtg agctagacga taagattaag    480
ggcgctatta ctgcccgggt gagaaaacta gatcatgaat taggtattcc agaagatgaa    540
actaagacaa ggggtaagtt tcacttttta aacagaatcc attacatggc accaagcaat    600
gaacctggg gtgaacatga aattgattac atcctatatt ataagatcaa cgctaaagaa    660
aacttgactg tcaacccaaa cgccaatgaa gttagagact tcaaatgggt ttcacccaat    720
gatttgaaaa ctatgtttgc tgaccaagt tacaagtta cgccttggt taagattatt    780
tgcgagaatt acttattcaa ctgggtgggag caattagatg acctttctga agtggaaaat    840
gacaggcaaa tcatagaat gctataa                                     867

```

<210> SEQ ID NO 36

<211> LENGTH: 1650

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: I. batatas IspS with C-terminal strepII tag
(underlined) for expression in T. reesei

<400> SEQUENCE: 36

```

atgactgccc gccgctcagc aaactatcaa cctctctcat ggtcttacga cgaataactg    60
gtggacacta ctactaacga cagcaaacgt cgcattcaag aagacgctcg taaaaaattg    120
gaagaagaag tgcgtaacgt tctggaagat ggcaaatggg aaaccttagc actggtggaa    180
ctgattgatg acatccaacg gctgggcttg ggttataaat ttcgcaaaag caccagtact    240
tccctggcta tgttgaaaat gagtgtgggg caggaagcat ccaacagcag tttgcattct    300
tgttcattgt actttcgttt actgcccggaa cacggcttcg atattacccc cgacgtgttc    360
gaaaaaattca aagatgaaaa cggtaaat taaagatagca tcgctaaaga tgttcgcccg    420
ttggtatcat tgatgaagc aagcttttta gggttcgaag gcgaaaacat tttggacgaa    480
gccccgcaat tcaccactat gcactctgaat aacatcaaa ataaagtgaa tccccgtatc    540
gcggaagaag ttaacatgc tttagaactg ccgttgacc gccgtgtgga acgtctggaa    600
gctcggcgcc gtattcaaag ctatagtaaa tccggtgaaa ccaatcaggg cctgctgacc    660
ctggctaaaa tcgattttaa caccgtgcag gcggtttacc aacgggatct gcaggacgtt    720
agtaaatggt ggaagacac tgcattggcc gataaattat ccttcgcccc ggatcgctg    780
atggaagct ttttctgggc gattggtatg agctatgatc cccaacactc taaatcacgg    840
gaagccgtga ccaaaacttt taaactggtg accgttttgg atgacgtgta tgacgtttac    900
gggtctttag atgaactgga aaaattcacc gccgcccggc aacgttggga tgttgacgcg    960
attaagatc tgccggacta catgaaattg tgttacttat ccctgtttaa tacctgaaac    1020
gatctggctt atgacacctt gaaagataaa ggcgaaactg ttattcctat catgaagaaa    1080

```

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```

gcttgggctg atttactgaa agcattttctg caggaagccc aatggatcta caacaaatac 1140
acccaactt tcgatgaata cctgaataac gcccgtttca gcgtgagtggtg ttgcgtgatg 1200
ttggttcata gctactttac cactcagaac atcaccaaag aagcgatcca ttctctggaa 1260
aactaccaog acttgtaaat ttggcctagc atcgttttcc gtttagcaaa tgatctgtcc 1320
tcttcaaaag ccgaaattga acggggcgaa accgcgaata gcatcacttg ttatatgaac 1380
gaaaccggtc aaagtgaaga acaggcccgt gaacacattt ccaaactgat cgatgaatgc 1440
ttcaagaaaa tgaacaaaga aatgctggcc acctccactt ctccgtttga aaaatccttc 1500
attgaaaccg cgatcaactt agcacgcatt gccctgtgcc agtatcaata cggcgatgcc 1560
catagcgcac cagatgttcg ggcacgcaac cgcattgtgt cagttatcat taatccagtg 1620
gaatgggtccc acccacaatt tgaaaagtaa 1650

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<210> SEQ ID NO 37

<211> LENGTH: 553

<212> TYPE: PRT

<213> ORGANISM: Quercus petraea

<400> SEQUENCE: 37

```

Met Thr Glu Arg Gln Ser Ala Asn Phe Gln Pro Ser Leu Trp Ser Tyr
1           5           10           15
Glu Tyr Ile Gln Ser Leu Lys Asn Gly Tyr Glu Ala Asp Leu Tyr Glu
20           25           30
Asp Arg Ala Lys Lys Leu Gly Glu Glu Val Arg Arg Met Ile Asn Asn
35           40           45
Lys Asp Thr Lys Leu Leu Thr Thr Leu Glu Leu Ile Asp Asp Ile Glu
50           55           60
Arg Leu Gly Leu Gly Tyr Arg Phe Lys Glu Glu Ile Met Arg Ala Leu
65           70           75           80
Asp Arg Phe Val Thr Leu Lys Gly Cys Glu Glu Phe Thr Asn Gly Ser
85           90           95
Ile His Asp Thr Ala Leu Ser Phe Arg Leu Leu Arg Gln His Gly Phe
100          105          110
Gly Val Ser Gln Asp Met Phe Asn Cys Phe Lys Asp Gln Lys Gly Asn
115          120          125
Phe Lys Glu Cys Leu Ser Lys Asp Ile Lys Gly Leu Leu Ser Leu Tyr
130          135          140
Glu Ala Ser Tyr Leu Gly Phe Glu Gly Glu Asn Leu Leu Asp Glu Ala
145          150          155          160
Arg Glu Phe Thr Thr Met His Leu Lys Asp Leu Lys Gly Asp Val Ser
165          170          175
Arg Thr Leu Lys Glu Glu Val Arg His Ser Leu Glu Met Pro Leu His
180          185          190
Arg Arg Met Arg Arg Leu Glu Gln Arg Trp Tyr Ile Asp Ala Tyr Asn
195          200          205
Met Lys Glu Ala His Asp Arg Lys Leu Leu Glu Leu Ala Lys Leu Asp
210          215          220
Phe Asn Ile Val Gln Ser Val His Gln Arg Asp Leu Lys Asp Met Ser
225          230          235          240
Arg Trp Trp Gln Glu Met Gly Leu Gly Asn Lys Leu Ser Phe Ala Arg
245          250          255
Asp Arg Leu Met Glu Cys Phe Phe Phe Ser Val Gly Met Ala Phe Glu
260          265          270

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Val Ile Glu Gln Ile Asp Thr Ala Trp Arg Gln Met Asn Lys Tyr Met
515 520 525

Val Asp His Ser Thr Phe Asn Arg Ser Phe Met Gln Met Thr Tyr Asn
530 535 540

Leu Ala Arg Met Ala His Cys Val Tyr Gln Asp Gly Asp Ala Ile Gly
545 550 555 560

Ala Pro Asp Asp Gln Ser Trp Asn Arg Val His Ser Leu Ile Ile Lys
565 570 575

Pro Val Ser Leu Ala Pro Cys
580

<210> SEQ ID NO 39
 <211> LENGTH: 582
 <212> TYPE: PRT
 <213> ORGANISM: Eucalyptus globulus

<400> SEQUENCE: 39

Met Ala Leu Arg Leu Leu Phe Thr Pro His Leu Pro Val Leu Ser Ser
1 5 10 15

Arg Arg Ala Asn Gly Arg Val Arg Cys Ser Ala Ser Thr Gln Ile Ser
20 25 30

Asp Pro Gln Glu Gly Arg Arg Ser Ala Asn Tyr Gln Pro Ser Val Trp
35 40 45

Thr Tyr Asn Tyr Leu Gln Ser Ile Val Ala Gly Glu Gly Arg Gln Ser
50 55 60

Arg Arg Glu Val Glu Gln Gln Lys Glu Lys Val Gln Ile Leu Glu Glu
65 70 75 80

Glu Val Arg Gly Ala Leu Asn Asp Glu Lys Ala Glu Thr Phe Thr Ile
85 90 95

Phe Ala Thr Val Asp Asp Ile Gln Arg Leu Gly Leu Gly Asp His Phe
100 105 110

Glu Glu Asp Ile Ser Asn Ala Leu Arg Arg Cys Val Ser Lys Gly Ala
115 120 125

Val Phe Met Ser Leu Gln Lys Ser Leu His Gly Thr Ala Leu Gly Phe
130 135 140

Arg Leu Leu Arg Gln His Gly Tyr Glu Val Ser Gln Asp Val Phe Lys
145 150 155 160

Ile Phe Leu Asp Glu Ser Gly Ser Phe Val Lys Thr Leu Gly Gly Asp
165 170 175

Val Gln Gly Val Leu Ser Leu Tyr Glu Ala Ser His Leu Ala Phe Glu
180 185 190

Glu Glu His Ile Leu His Lys Ala Arg Ser Phe Ala Ile Lys His Leu
195 200 205

Glu Asn Leu Asn Ser Asp Val Asp Lys Asp Leu Gln Asp Gln Val Lys
210 215 220

His Glu Leu Glu Leu Pro Leu His Arg Arg Met Pro Leu Leu Glu Ala
225 230 235 240

Arg Arg Ser Ile Glu Ala Tyr Ser Arg Arg Gly Tyr Thr Asn Pro Gln
245 250 255

Ile Leu Glu Leu Ala Leu Thr Asp Phe Asn Val Ser Gln Ser Tyr Leu
260 265 270

Gln Arg Asp Leu Gln Glu Met Leu Gly Trp Trp Asn Asn Thr Gly Leu
275 280 285

Ala Lys Arg Leu Ser Phe Ala Arg Asp Arg Leu Ile Glu Cys Phe Phe
290 295 300

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Trp Ala Val Gly Ile Ala His Glu Pro Ser Leu Ser Ile Cys Arg Lys
 305 310 315 320
 Ala Val Thr Lys Ala Phe Ala Leu Ile Leu Val Leu Asp Asp Val Tyr
 325 330 335
 Asp Val Phe Gly Thr Leu Glu Glu Leu Glu Leu Phe Thr Asp Ala Val
 340 345 350
 Arg Arg Trp Asp Leu Asn Ala Val Glu Asp Leu Pro Val Tyr Met Lys
 355 360 365
 Leu Cys Tyr Leu Ala Leu Tyr Asn Ser Val Asn Glu Met Ala Tyr Glu
 370 375 380
 Thr Leu Lys Glu Lys Gly Glu Asn Val Ile Pro Tyr Leu Ala Lys Ala
 385 390 395 400
 Trp Tyr Asp Leu Cys Lys Ala Phe Leu Gln Glu Ala Lys Trp Ser Asn
 405 410 415
 Ser Arg Ile Ile Pro Gly Val Glu Glu Tyr Leu Asn Asn Gly Trp Val
 420 425 430
 Ser Ser Ser Gly Ser Val Met Leu Ile His Ala Tyr Phe Leu Ala Ser
 435 440 445
 Pro Ser Ile Arg Lys Glu Glu Leu Glu Ser Leu Glu His Tyr His Asp
 450 455 460
 Leu Leu Arg Leu Pro Ser Leu Ile Phe Arg Leu Thr Asn Asp Ile Ala
 465 470 475 480
 Ser Ser Ser Ala Glu Leu Glu Arg Gly Glu Thr Thr Asn Ser Ile Arg
 485 490 495
 Cys Phe Met Gln Glu Lys Gly Ile Ser Glu Leu Glu Ala Arg Glu Cys
 500 505 510
 Val Lys Glu Glu Ile Asp Thr Ala Trp Lys Lys Met Asn Lys Tyr Met
 515 520 525
 Val Asp Arg Ser Thr Phe Asn Gln Ser Phe Val Arg Met Thr Tyr Asn
 530 535 540
 Leu Ala Arg Met Ala His Cys Val Tyr Gln Asp Gly Asp Ala Ile Gly
 545 550 555 560
 Ser Pro Asp Asp Leu Ser Trp Asn Arg Val His Ser Leu Ile Ile Lys
 565 570 575
 Pro Ile Ser Pro Ala Ala
 580

<210> SEQ ID NO 40
 <211> LENGTH: 595
 <212> TYPE: PRT
 <213> ORGANISM: Populus alba

<400> SEQUENCE: 40

Met Ala Thr Glu Leu Leu Cys Leu His Arg Pro Ile Ser Leu Thr His
 1 5 10 15
 Lys Leu Phe Arg Asn Pro Leu Pro Lys Val Ile Gln Ala Thr Pro Leu
 20 25 30
 Thr Leu Lys Leu Arg Cys Ser Val Ser Thr Glu Asn Val Ser Phe Thr
 35 40 45
 Glu Thr Glu Thr Glu Ala Arg Arg Ser Ala Asn Tyr Glu Pro Asn Ser
 50 55 60
 Trp Asp Tyr Asp Tyr Leu Leu Ser Ser Asp Thr Asp Glu Ser Ile Glu
 65 70 75 80
 Val Tyr Lys Asp Lys Ala Lys Lys Leu Glu Ala Glu Val Arg Arg Glu

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85					90					95					
Ile	Asn	Asn	Glu	Lys	Ala	Glu	Phe	Leu	Thr	Leu	Leu	Glu	Leu	Ile	Asp
			100					105						110	
Asn	Val	Gln	Arg	Leu	Gly	Leu	Gly	Tyr	Arg	Phe	Glu	Ser	Asp	Ile	Arg
		115					120						125		
Gly	Ala	Leu	Asp	Arg	Phe	Val	Ser	Ser	Gly	Gly	Phe	Asp	Ala	Val	Thr
	130					135						140			
Lys	Thr	Ser	Leu	His	Gly	Thr	Ala	Leu	Ser	Phe	Arg	Leu	Leu	Arg	Gln
145					150						155				160
His	Gly	Phe	Glu	Val	Ser	Gln	Glu	Ala	Phe	Ser	Gly	Phe	Lys	Asp	Gln
				165					170						175
Asn	Gly	Asn	Phe	Leu	Glu	Asn	Leu	Lys	Glu	Asp	Ile	Lys	Ala	Ile	Leu
			180					185						190	
Ser	Leu	Tyr	Glu	Ala	Ser	Phe	Leu	Ala	Leu	Glu	Gly	Glu	Asn	Ile	Leu
		195					200						205		
Asp	Glu	Ala	Lys	Val	Phe	Ala	Ile	Ser	His	Leu	Lys	Glu	Leu	Ser	Glu
	210					215						220			
Glu	Lys	Ile	Gly	Lys	Glu	Leu	Ala	Glu	Gln	Val	Asn	His	Ala	Leu	Glu
225					230					235					240
Leu	Pro	Leu	His	Arg	Arg	Thr	Gln	Arg	Leu	Glu	Ala	Val	Trp	Ser	Ile
				245					250						255
Glu	Ala	Tyr	Arg	Lys	Lys	Glu	Asp	Ala	Asn	Gln	Val	Leu	Leu	Glu	Leu
			260					265						270	
Ala	Ile	Leu	Asp	Tyr	Asn	Met	Ile	Gln	Ser	Val	Tyr	Gln	Arg	Asp	Leu
		275					280						285		
Arg	Glu	Thr	Ser	Arg	Trp	Trp	Arg	Arg	Val	Gly	Leu	Ala	Thr	Lys	Leu
	290					295					300				
His	Phe	Ala	Arg	Asp	Arg	Leu	Ile	Glu	Ser	Phe	Tyr	Trp	Ala	Val	Gly
305					310						315				320
Val	Ala	Phe	Glu	Pro	Gln	Tyr	Ser	Asp	Cys	Arg	Asn	Ser	Val	Ala	Lys
				325					330						335
Met	Phe	Ser	Phe	Val	Thr	Ile	Ile	Asp	Asp	Ile	Tyr	Asp	Val	Tyr	Gly
			340					345						350	
Thr	Leu	Asp	Glu	Leu	Glu	Leu	Phe	Thr	Asp	Ala	Val	Glu	Arg	Trp	Asp
		355					360						365		
Val	Asn	Ala	Ile	Asn	Asp	Leu	Pro	Asp	Tyr	Met	Lys	Leu	Cys	Phe	Leu
	370					375					380				
Ala	Leu	Tyr	Asn	Thr	Ile	Asn	Glu	Ile	Ala	Tyr	Asp	Asn	Leu	Lys	Asp
385				390						395					400
Lys	Gly	Glu	Asn	Ile	Leu	Pro	Tyr	Leu	Thr	Lys	Ala	Trp	Ala	Asp	Leu
				405					410						415
Cys	Asn	Ala	Phe	Leu	Gln	Glu	Ala	Lys	Trp	Leu	Tyr	Asn	Lys	Ser	Thr
			420					425						430	
Pro	Thr	Phe	Asp	Asp	Tyr	Phe	Gly	Asn	Ala	Trp	Lys	Ser	Ser	Ser	Gly
			435				440						445		
Pro	Leu	Gln	Leu	Val	Phe	Ala	Tyr	Phe	Ala	Val	Val	Gln	Asn	Ile	Lys
	450					455						460			
Lys	Glu	Glu	Ile	Glu	Asn	Leu	Gln	Lys	Tyr	His	Asp	Thr	Ile	Ser	Arg
465					470					475					480
Pro	Ser	His	Ile	Phe	Arg	Leu	Cys	Asn	Asp	Leu	Ala	Ser	Ala	Ser	Ala
			485						490						495
Glu	Ile	Ala	Arg	Gly	Glu	Thr	Ala	Asn	Ser	Val	Ser	Cys	Tyr	Met	Arg
			500					505							510

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275	280	285
Arg Glu Thr Ser Arg Trp Trp Arg Arg Val Gly Leu Ala Thr Lys Leu		
290	295	300
His Phe Ala Lys Asp Arg Leu Ile Glu Ser Phe Tyr Trp Ala Val Gly		
305	310	315
Val Ala Phe Glu Pro Gln Tyr Ser Asp Cys Arg Asn Ser Val Ala Lys		
	325	330
Met Phe Ser Phe Val Thr Ile Ile Asp Asp Ile Tyr Asp Val Tyr Gly		
	340	345
Thr Leu Asp Glu Leu Glu Leu Phe Thr Asp Ala Val Glu Arg Trp Asp		
	355	360
Val Asn Ala Ile Asn Asp Leu Pro Asp Tyr Met Lys Leu Cys Phe Leu		
	370	375
Ala Leu Tyr Asn Thr Ile Asn Glu Ile Ala Tyr Asp Asn Leu Lys Asp		
	385	390
Lys Gly Glu Asn Ile Leu Pro Tyr Leu Thr Lys Ala Trp Ala Asp Leu		
	405	410
Cys Asn Ala Phe Leu Gln Glu Ala Lys Trp Leu Tyr Asn Lys Ser Thr		
	420	425
Pro Thr Phe Asp Asp Tyr Phe Gly Asn Ala Trp Lys Ser Ser Ser Gly		
	435	440
Pro Leu Gln Leu Ile Phe Ala Tyr Phe Ala Val Val Gln Asn Ile Lys		
	450	455
Lys Glu Glu Ile Glu Asn Leu Gln Lys Tyr His Asp Ile Ile Ser Arg		
	465	470
Pro Ser His Ile Phe Arg Leu Cys Asn Asp Leu Ala Ser Ala Ser Ala		
	485	490
Glu Ile Ala Arg Gly Glu Thr Ala Asn Ser Val Ser Cys Tyr Met Arg		
	500	505
Thr Lys Gly Ile Ser Glu Glu Leu Ala Thr Glu Ser Val Met Asn Leu		
	515	520
Ile Asp Glu Thr Cys Lys Lys Met Asn Lys Glu Lys Leu Gly Gly Ser		
	530	535
Leu Phe Ala Lys Pro Phe Val Glu Thr Ala Ile Asn Leu Ala Arg Gln		
	545	550
Ser His Cys Thr Tyr His Asn Gly Asp Ala His Thr Ser Pro Asp Glu		
	565	570
Leu Thr Arg Lys Arg Val Leu Ser Val Ile Thr Glu Pro Ile Leu Pro		
	580	585
		590
Phe Glu Arg		
595		

<210> SEQ ID NO 42
 <211> LENGTH: 595
 <212> TYPE: PRT
 <213> ORGANISM: Salix sp.

<400> SEQUENCE: 42

Met Ala Thr Glu Leu Leu Cys Leu His Arg Pro Ile Ser Leu Thr Pro
1 5 10 15
Lys Leu Phe Arg Asn Pro Leu Pro Lys Val Ile Leu Ala Thr Pro Leu
20 25 30
Thr Leu Lys Leu Arg Cys Ser Val Ser Thr Glu Asn Val Ser Phe Thr
35 40 45

-continued

Glu Thr Glu Thr Glu Thr Arg Arg Ser Ala Asn Tyr Glu Pro Asn Ser
 50 55 60
 Trp Asp Tyr Asp Tyr Leu Leu Ser Ser Asp Thr Asp Glu Ser Ile Glu
 65 70 75 80
 Val Tyr Lys Asp Lys Ala Lys Lys Leu Glu Ala Glu Val Arg Arg Glu
 85 90 95
 Ile Asn Asn Glu Lys Ala Glu Phe Leu Thr Leu Leu Glu Leu Ile Asp
 100 105 110
 Asn Val Gln Arg Leu Gly Leu Gly Tyr Arg Phe Glu Ser Asp Ile Arg
 115 120 125
 Arg Ala Leu Asp Arg Phe Val Ser Ser Gly Gly Phe Asp Ala Val Thr
 130 135 140
 Lys Thr Ser Leu His Ala Thr Ala Leu Ser Phe Arg Phe Leu Arg Gln
 145 150 155 160
 His Gly Phe Glu Val Ser Gln Glu Ala Phe Gly Gly Phe Lys Asp Gln
 165 170 175
 Asn Gly Asn Phe Leu Glu Asn Leu Lys Glu Asp Ile Lys Ala Ile Leu
 180 185 190
 Ser Leu Tyr Glu Ala Ser Phe Leu Ala Leu Glu Gly Glu Asn Ile Leu
 195 200 205
 Asp Glu Ala Lys Val Phe Ala Ile Ser His Leu Lys Glu Leu Ser Glu
 210 215 220
 Glu Lys Ile Gly Lys Asp Leu Ala Glu Gln Val Asn His Ala Leu Glu
 225 230 235 240
 Leu Pro Leu His Arg Arg Thr Gln Arg Leu Glu Ala Val Trp Ser Ile
 245 250 255
 Glu Ala Tyr Arg Lys Lys Glu Asp Ala Asn Gln Val Leu Leu Glu Leu
 260 265 270
 Ala Ile Leu Asp Tyr Asn Met Ile Gln Ser Val Tyr Gln Arg Asp Leu
 275 280 285
 Arg Glu Thr Ser Arg Trp Trp Arg Arg Val Gly Leu Ala Thr Lys Leu
 290 295 300
 His Phe Ala Arg Asp Arg Leu Ile Glu Ser Phe Tyr Trp Ala Val Gly
 305 310 315 320
 Val Ala Phe Glu Pro Gln Tyr Ser Asp Cys Arg Asn Ser Val Ala Lys
 325 330 335
 Met Phe Ser Phe Val Thr Ile Ile Asp Asp Ile Tyr Asp Val Tyr Gly
 340 345 350
 Thr Leu Asp Glu Leu Glu Leu Phe Thr Asp Ala Val Glu Arg Trp Asp
 355 360 365
 Val Asn Ala Ile Asn Asp Leu Pro Asp Tyr Met Lys Leu Cys Phe Leu
 370 375 380
 Ala Leu Tyr Asn Thr Ile Asn Glu Ile Ala Tyr Asp Asn Leu Lys Glu
 385 390 395 400
 Lys Gly Glu Asn Ile Leu Pro Tyr Leu Thr Lys Ala Trp Ala Asp Leu
 405 410 415
 Cys Asn Ala Phe Leu Gln Glu Ala Lys Trp Leu Tyr Asn Lys Ser Thr
 420 425 430
 Pro Thr Phe Asp Asp Tyr Phe Gly Asn Ala Trp Lys Ser Ser Ser Gly
 435 440 445
 Pro Leu Gln Leu Val Phe Ala Tyr Phe Ala Val Val Gln Asn Ile Lys
 450 455 460
 Lys Glu Glu Ile Glu Asn Leu Gln Lys Tyr His Asp Ile Ile Ser Arg

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Ile Asn Met Ala Arg Val Ser His Cys Thr Tyr Gln Tyr Gly Asp Gly
    500                               505          510
Leu Gly Arg Pro Asp Asn Thr Ala Glu Asn Arg Ile Lys Leu Leu Leu
    515                               520          525
Ile Asp Pro Phe Pro Ile
    530

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<210> SEQ ID NO 45
<211> LENGTH: 544
<212> TYPE: PRT
<213> ORGANISM: Arachis hypogaea

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<400> SEQUENCE: 45

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Met Asn Thr Arg Arg Ser Ala Asn Tyr Gln Pro Asn Leu Trp Asp Phe
 1      5      10      15
Glu Phe Leu Gln Ser Val Glu Asn Asp Leu Gln Val Glu Arg Leu Glu
 20      25      30
Glu Arg Ala Arg Lys Leu Glu Glu Glu Val Arg Gly Leu Met Lys Lys
 35      40      45
Val Glu Ile Glu Pro Leu Ser Leu Leu Glu Leu Met Asp Asn Val Glu
 50      55      60
Arg Leu Gly Leu Thr Tyr Lys Phe Glu Glu Asp Ile Lys Ser Ala Leu
 65      70      75      80
Asn Asn Arg Ile Val Pro Leu Leu His His His Thr Ile Asn Lys Tyr
 85      90      95
Gly Leu His Ala Thr Ala Leu Ser Phe Arg Phe Leu Arg Gln His Ala
 100     105     110
Phe His Val Ser Pro Asp Val Phe Glu Ser Phe Lys Glu Glu Gly Lys
 115     120     125
Phe Lys Lys Glu Ile Ser Gly Asp Val Leu Gly Leu Leu Asn Leu Tyr
 130     135     140
Glu Thr Ser Tyr Leu Gly Phe Glu Gly Glu Thr Ile Leu Asp Glu Ala
 145     150     155     160
Arg Ala Phe Ser Ala Thr His Leu Lys Asn Leu Leu Gln Thr Asn Gln
 165     170     175
Val Gln Asn Lys Val Met Ala Glu Lys Val Arg His Ala Leu Glu Leu
 180     185     190
Pro Tyr His Arg Arg Val His Arg Leu Glu Ala Arg Trp Phe Ile Glu
 195     200     205
Arg Tyr Glu Gln Lys Glu Ala His Asp Gly Ala Leu Leu Glu Leu Ala
 210     215     220
Lys Leu Asp Phe Asn Met Val Gln Ser Val Met Lys Lys Glu Leu Gln
 225     230     235     240
Glu Leu Ser Arg Trp Trp Arg Glu Ile Gly Leu Thr Ser Lys Leu Asp
 245     250     255
Phe Val Arg Asp Arg Leu Met Glu Val Tyr Phe Trp Ala Leu Gly Met
 260     265     270
Ala Pro His Pro Gln Leu Thr Glu Cys Arg Lys Ala Val Thr Lys Met
 275     280     285
Phe Gly Leu Val Thr Ile Ile Asp Asp Val Tyr Asp Val Tyr Gly Thr
 290     295     300
Leu Asp Glu Leu Gln Leu Phe Thr Asp Ala Val Asp Arg Trp Asp Val
 305     310     315     320
Asn Ala Val Glu Thr Leu Pro Asp Tyr Met Lys Leu Cys Tyr Leu Ala
 325     330     335

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Leu Tyr Asn Ser Val Asn Asp Thr Ala Tyr Ser Thr Leu Arg Glu Lys
 340 345 350
 Gly Asp Asn Ser Leu Pro His Leu Ala Lys Ser Trp Arg Asp Leu Cys
 355 360 365
 Lys Ala Phe Leu Gln Glu Ala Lys Trp Ser Asn Asn Lys Ile Ile Pro
 370 375 380
 Pro Phe Asp Ala Tyr Ile Arg Asn Ala Ser Val Ser Ser Ser Gly Gly
 385 390 395 400
 Ala Leu Leu Ala Pro Cys Tyr Phe Ser Val Thr Gln Asp Ser Thr Ser
 405 410 415
 Gln Ala Ile Asp Ser Ile Thr Asn Tyr His Gly Ile Val Arg Ser Ser
 420 425 430
 Cys Ala Ile Phe Arg Leu Cys Asn Asp Leu Ala Thr Ser Ala Ala Glu
 435 440 445
 Leu Glu Arg Gly Glu Thr Thr Asn Ser Ile Thr Ser Tyr Met Thr Glu
 450 455 460
 Asn Gly Thr Thr Glu Glu Glu Ala Arg Glu Ser Leu Gly Lys Leu Ile
 465 470 475 480
 Asp Gln Glu Trp Lys Lys Met Asn Arg Asp Val Val Leu Glu Ser Ala
 485 490 495
 Tyr Pro Asn Val Phe Lys Glu Ile Ala Ile Asn Met Ala Arg Val Ser
 500 505 510
 His Cys Thr Tyr Gln Tyr Gly Asp Gly Leu Gly Arg Pro Asp Asp Thr
 515 520 525
 Ala Glu Asn Arg Ile Lys Leu Ser Leu Ile Glu Pro Ile Pro Ile Asn
 530 535 540

<210> SEQ ID NO 46
 <211> LENGTH: 545
 <212> TYPE: PRT
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 46

Met Glu Thr Arg Arg Ser Ala Asn Tyr Gln Pro Asn Leu Trp Asn Phe
 1 5 10 15
 Glu Phe Leu Pro Pro Ser Leu Glu Asn Asp His Lys Val Glu Lys Leu
 20 25 30
 Glu Glu Arg Ala Lys Lys Val Glu Glu Glu Val Arg Lys Val Ile Asn
 35 40 45
 Gly Ile Asp Thr Lys Pro Leu Leu Leu Glu Leu Ile Asp Asp Val Gln
 50 55 60
 His Leu Gly Leu Thr Tyr Lys Phe Glu Lys Asp Ile Ile Lys Ala Leu
 65 70 75 80
 Glu Lys Ile Val Ser Leu Asp Glu Asn Glu Glu His Lys Ser Glu Leu
 85 90 95
 Tyr Tyr Thr Ala Leu Ser Phe Arg Leu Leu Arg Gln His Gly Phe Glu
 100 105 110
 Val Ser Gln Asp Val Phe Lys Arg Phe Lys Asp Lys Glu Gly Gly Phe
 115 120 125
 Ser Gly Glu Leu Lys Gly Asp Val Gln Gly Leu Leu Ser Leu Tyr Glu
 130 135 140
 Ala Ser Tyr Leu Gly Phe Glu Gly Asp Asn Leu Leu Asp Glu Ala Arg
 145 150 155 160
 Ala Phe Ser Thr Thr His Leu Lys Asn Asn Leu Lys Gln Gly Ile Asn

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165					170					175					
Thr	Lys	Glu	Ala	Glu	Gln	Val	Asn	His	Ala	Leu	Glu	Leu	Pro	Tyr	His
			180						185					190	
Arg	Arg	Leu	Gln	Arg	Leu	Glu	Ala	Arg	Trp	Tyr	Leu	Glu	Lys	Tyr	Glu
		195					200						205		
Pro	Lys	Glu	Pro	His	His	Gln	Leu	Leu	Leu	Glu	Leu	Ala	Lys	Leu	Asp
	210					215						220			
Phe	Asn	Met	Val	Gln	Leu	Leu	His	Gln	Lys	Glu	Leu	Gln	Glu	Leu	Ser
225					230					235					240
Arg	Trp	Trp	Ser	Glu	Met	Gly	Leu	Ala	Ser	Lys	Leu	Glu	Phe	Ala	Arg
			245						250						255
Asp	Arg	Leu	Met	Glu	Val	Tyr	Phe	Trp	Ala	Leu	Gly	Met	Ala	Pro	Asp
		260						265						270	
Pro	Gln	Phe	Arg	Glu	Cys	Arg	Lys	Ala	Val	Thr	Lys	Met	Phe	Gly	Leu
		275					280						285		
Val	Thr	Ile	Ile	Asp	Asp	Val	Tyr	Asp	Ile	Tyr	Gly	Thr	Leu	Asp	Glu
	290					295					300				
Leu	Gln	Leu	Phe	Thr	Asp	Ala	Val	Glu	Arg	Trp	Asp	Val	Asn	Val	Val
305					310					315					320
Asn	Thr	Leu	Pro	Asp	Tyr	Met	Lys	Leu	Cys	Tyr	Leu	Ala	Leu	Tyr	Asn
			325						330						335
Thr	Val	Asn	Asp	Thr	Ala	Tyr	Ser	Ile	Leu	Lys	Glu	Lys	Gly	Arg	Asn
		340						345						350	
Asn	Leu	Ser	Tyr	Leu	Lys	Lys	Ser	Trp	Cys	Glu	Leu	Cys	Lys	Ala	Phe
		355					360						365		
Leu	Gln	Glu	Ala	Lys	Trp	Ser	Asn	Asn	Lys	Ile	Val	Pro	Ala	Phe	Ser
	370					375					380				
Lys	Tyr	Leu	Glu	Asn	Ala	Ser	Val	Ser	Ser	Ser	Gly	Val	Ala	Leu	Leu
385					390					395					400
Ala	Pro	Ser	Tyr	Phe	Ser	Val	Cys	Gln	Glu	Gln	Asp	Ile	Ser	Phe	Ser
			405						410						415
Asp	Lys	Thr	Leu	His	Tyr	Leu	Thr	Asn	Phe	Gly	Gly	Leu	Val	Arg	Ser
		420						425						430	
Ser	Cys	Thr	Ile	Phe	Arg	Leu	Cys	Asn	Asp	Leu	Thr	Thr	Ser	Ala	Ala
		435					440						445		
Glu	Leu	Glu	Arg	Gly	Glu	Thr	Thr	Asn	Ser	Ile	Met	Ser	Tyr	Met	His
	450					455					460				
Glu	Asn	Gly	Thr	Ser	Glu	Glu	His	Ala	Cys	Glu	Glu	Leu	Arg	Asn	Leu
465					470					475					480
Ile	Asp	Ile	Glu	Trp	Lys	Lys	Met	Asn	Arg	Gln	Arg	Val	Ser	Asp	Ser
			485						490						495
Thr	Leu	Pro	Lys	Ala	Phe	Arg	Glu	Ile	Ala	Met	Asn	Met	Ala	Arg	Val
		500						505						510	
Ser	His	Asn	Thr	Tyr	Gln	Tyr	Gly	Asp	Gly	Leu	Gly	Arg	Pro	Asp	Tyr
		515					520					525			
Asn	Ile	Glu	Asn	Arg	Ile	Lys	Phe	Leu	Leu	Ile	Asp	Pro	Val	Pro	Ile
	530					535					540				

Asn
545

<210> SEQ ID NO 47
 <211> LENGTH: 608
 <212> TYPE: PRT
 <213> ORGANISM: Pueraria montana var. lobata

-continued

<400> SEQUENCE: 47

Met Ala Thr Asn Leu Leu Cys Leu Ser Asn Lys Leu Ser Ser Pro Thr
 1 5 10 15
 Pro Thr Pro Ser Thr Arg Phe Pro Gln Ser Lys Asn Phe Ile Thr Gln
 20 25 30
 Lys Thr Ser Leu Ala Asn Pro Lys Pro Trp Arg Val Ile Cys Ala Thr
 35 40 45
 Ser Ser Gln Phe Thr Gln Ile Thr Glu His Asn Ser Arg Arg Ser Ala
 50 55 60
 Asn Tyr Gln Pro Asn Leu Trp Asn Phe Glu Phe Leu Gln Ser Leu Glu
 65 70 75 80
 Asn Asp Leu Lys Val Glu Lys Leu Glu Glu Lys Ala Thr Lys Leu Glu
 85 90 95
 Glu Glu Val Arg Cys Met Ile Asn Arg Val Asp Thr Gln Pro Leu Ser
 100 105 110
 Leu Leu Glu Leu Ile Asp Asp Val Gln Arg Leu Gly Leu Thr Tyr Lys
 115 120 125
 Phe Glu Lys Asp Ile Ile Lys Ala Leu Glu Asn Ile Val Leu Leu Asp
 130 135 140
 Glu Asn Lys Lys Asn Lys Ser Asp Leu His Ala Thr Ala Leu Ser Phe
 145 150 155 160
 Arg Leu Leu Arg Gln His Gly Phe Glu Val Ser Gln Asp Val Phe Glu
 165 170 175
 Arg Phe Lys Asp Lys Glu Gly Gly Phe Ser Gly Glu Leu Lys Gly Asp
 180 185 190
 Val Gln Gly Leu Leu Ser Leu Tyr Glu Ala Ser Tyr Leu Gly Phe Glu
 195 200 205
 Gly Glu Asn Leu Leu Glu Glu Ala Arg Thr Phe Ser Ile Thr His Leu
 210 215 220
 Lys Asn Asn Leu Lys Glu Gly Ile Asn Thr Lys Val Ala Glu Gln Val
 225 230 235 240
 Ser His Ala Leu Glu Leu Pro Tyr His Gln Arg Leu His Arg Leu Glu
 245 250 255
 Ala Arg Trp Phe Leu Asp Lys Tyr Glu Pro Lys Glu Pro His His Gln
 260 265 270
 Leu Leu Leu Glu Leu Ala Lys Leu Asp Phe Asn Met Val Gln Thr Leu
 275 280 285
 His Gln Lys Glu Leu Gln Asp Leu Ser Arg Trp Trp Thr Glu Met Gly
 290 295 300
 Leu Ala Ser Lys Leu Asp Phe Val Arg Asp Arg Leu Met Glu Val Tyr
 305 310 315 320
 Phe Trp Ala Leu Gly Met Ala Pro Asp Pro Gln Phe Gly Glu Cys Arg
 325 330 335
 Lys Ala Val Thr Lys Met Phe Gly Leu Val Thr Ile Ile Asp Asp Val
 340 345 350
 Tyr Asp Val Tyr Gly Thr Leu Asp Glu Leu Gln Leu Phe Thr Asp Ala
 355 360 365
 Val Glu Arg Trp Asp Val Asn Ala Ile Asn Thr Leu Pro Asp Tyr Met
 370 375 380
 Lys Leu Cys Phe Leu Ala Leu Tyr Asn Thr Val Asn Asp Thr Ser Tyr
 385 390 395 400
 Ser Ile Leu Lys Glu Lys Gly His Asn Asn Leu Ser Tyr Leu Thr Lys

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405					410					415					
Ser	Trp	Arg	Glu	Leu	Cys	Lys	Ala	Phe	Leu	Gln	Glu	Ala	Lys	Trp	Ser
			420					425					430		
Asn	Asn	Lys	Ile	Ile	Pro	Ala	Phe	Ser	Lys	Tyr	Leu	Glu	Asn	Ala	Ser
		435					440					445			
Val	Ser	Ser	Ser	Gly	Val	Ala	Leu	Leu	Ala	Pro	Ser	Tyr	Phe	Ser	Val
	450					455					460				
Cys	Gln	Gln	Gln	Glu	Asp	Ile	Ser	Asp	His	Ala	Leu	Arg	Ser	Leu	Thr
465					470					475					480
Asp	Phe	His	Gly	Leu	Val	Arg	Ser	Ser	Cys	Val	Ile	Phe	Arg	Leu	Cys
				485					490						495
Asn	Asp	Leu	Ala	Thr	Ser	Ala	Ala	Glu	Leu	Glu	Arg	Gly	Glu	Thr	Thr
		500						505					510		
Asn	Ser	Ile	Ile	Ser	Tyr	Met	His	Glu	Asn	Asp	Gly	Thr	Ser	Glu	Glu
		515					520					525			
Gln	Ala	Arg	Glu	Glu	Leu	Arg	Lys	Leu	Ile	Asp	Ala	Glu	Trp	Lys	Lys
	530					535					540				
Met	Asn	Arg	Glu	Arg	Val	Ser	Asp	Ser	Thr	Leu	Leu	Pro	Lys	Ala	Phe
545					550					555					560
Met	Glu	Ile	Ala	Val	Asn	Met	Ala	Arg	Val	Ser	His	Cys	Thr	Tyr	Gln
				565					570						575
Tyr	Gly	Asp	Gly	Leu	Gly	Arg	Pro	Asp	Tyr	Ala	Thr	Glu	Asn	Arg	Ile
			580					585					590		
Lys	Leu	Leu	Leu	Ile	Asp	Pro	Phe	Pro	Ile	Asn	Gln	Leu	Met	Tyr	Val
		595					600					605			

<210> SEQ ID NO 48

<211> LENGTH: 600

<212> TYPE: PRT

<213> ORGANISM: Mucuna pruriens

<400> SEQUENCE: 48

Met	Ala	Thr	Lys	Val	Leu	Cys	Leu	Ser	Asn	Gln	Phe	Leu	Tyr	Pro	Thr
1				5					10					15	
Pro	Thr	Leu	Thr	Ser	Thr	Arg	Phe	Leu	Gln	Thr	Glu	Asn	Phe	Thr	Gln
			20					25					30		
Lys	Thr	Ser	Leu	Ile	Asn	Pro	Lys	Pro	Tyr	Pro	Leu	Phe	Cys	Val	Val
			35			40						45			
Thr	Ser	Gln	Phe	Ser	Gln	Ile	Thr	Glu	Asp	Asn	Thr	Arg	Arg	Ser	Ala
			50			55					60				
Asn	Tyr	His	Pro	Asn	Leu	Trp	Asn	Phe	Glu	Phe	Leu	Gln	Ser	Leu	Glu
65					70					75					80
Asn	Asp	Pro	Lys	Ile	Glu	Lys	Leu	Glu	Glu	Lys	Ala	Thr	Lys	Leu	Val
				85					90					95	
Glu	Glu	Val	Arg	His	Met	Met	Asn	Lys	Ala	Glu	Thr	Glu	Pro	Leu	Ser
			100					105					110		
Leu	Leu	Glu	Leu	Ile	Asp	Asp	Val	Gln	Arg	Leu	Gly	Leu	Thr	Tyr	Lys
			115				120					125			
Phe	Glu	Lys	Asp	Ile	Ile	Asn	Ala	Leu	Glu	Lys	Thr	Ile	Ser	Leu	Asp
			130				135					140			
Glu	Asn	Gln	Lys	His	Ile	Ser	Gly	Leu	His	Ala	Thr	Ser	Leu	Ser	Phe
145					150					155					160
Arg	Leu	Leu	Arg	Gln	His	Gly	Phe	Glu	Val	Ser	Gln	Asp	Val	Phe	Lys
				165					170						175

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Lys Phe Lys Asp Glu Asp Gly Gly Phe Ser Ala Glu Leu Lys Gly Asp
 180 185 190
 Val Gln Gly Leu Leu Ser Leu Tyr Glu Ala Ser Tyr Leu Gly Phe Glu
 195 200 205
 Gly Glu Asn Leu Leu Asp Glu Ala Arg Glu Phe Ser Ile Glu His Leu
 210 215 220
 Lys Asn Asn Leu Asn Lys Gly Ile Thr Thr Lys Val Ala Glu Gln Val
 225 230 235 240
 Ser His Ala Leu Glu Leu Pro Tyr His Arg Arg Ile His Arg Leu Glu
 245 250 255
 Ala Arg Trp Phe Leu Asp Lys Tyr Glu Pro Lys Glu Ser Gln His Lys
 260 265 270
 Leu Leu Leu Glu Leu Ala Lys Leu Asp Phe Asn Met Val Gln Ser Leu
 275 280 285
 His Gln Lys Glu Leu Arg Glu Leu Ser Met Trp Trp Arg Glu Ile Gly
 290 295 300
 Leu Thr Ser Lys Leu Asp Phe Val Arg Asp Arg Leu Met Glu Val Tyr
 305 310 315 320
 Phe Trp Ala Leu Gly Met Ala Pro Asp Pro Gln Phe Ser Glu Cys Arg
 325 330 335
 Lys Ala Val Thr Lys Met Phe Gly Leu Val Thr Ile Ile Asp Asp Val
 340 345 350
 Tyr Asp Val Tyr Gly Thr Leu Asp Glu Leu Gln Leu Phe Thr Asp Ala
 355 360 365
 Val Glu Arg Trp Asp Val Asn Ala Ile Asn Thr Leu Pro Asp Tyr Met
 370 375 380
 Lys Leu Cys Phe Leu Ala Leu Tyr Asn Thr Val Asn Asp Thr Thr Tyr
 385 390 395 400
 Ser Ile Leu Lys Glu Lys Gly His Asn Asn Ile Ser Tyr Leu Thr Lys
 405 410 415
 Ser Trp Cys Glu Leu Cys Lys Ala Phe Leu Gln Glu Ala Lys Trp Ser
 420 425 430
 Asn Asn Lys Ile Ile Pro Thr Phe Asn Lys Tyr Leu Arg Asn Ala Ser
 435 440 445
 Val Ser Ser Ser Gly Val Ala Leu Leu Ala Pro Ser Phe Phe Leu Val
 450 455 460
 Cys Gln Glu Gln Asp Ile Ser Glu Gln Ala Leu His Ser Leu Ile Asn
 465 470 475 480
 Phe His Gly Leu Val Arg Ser Ser Cys Val Ile Phe Arg Leu Cys Asn
 485 490 495
 Asp Leu Ala Thr Ser Ala Ala Glu Leu Glu Arg Gly Glu Thr Thr Asn
 500 505 510
 Ser Ile Thr Ser Tyr Met His Glu Asn Gly Thr Ser Glu Glu Gln Ala
 515 520 525
 Arg Gln Glu Leu Arg Ile Leu Ile Asp Ala Glu Trp Lys Asn Met Asn
 530 535 540
 Gln Glu Arg Tyr Leu Asp Ser Thr Leu Pro Asp Ala Phe Met Glu Ile
 545 550 555 560
 Thr Ile Asn Leu Ala Arg Val Ser His Cys Thr Tyr Gln Tyr Gly Asp
 565 570 575
 Gly Leu Gly Arg Pro Asp Tyr Thr Thr Lys Asn Arg Ile Lys Leu Leu
 580 585 590
 Leu Ile Asp Pro Leu Pro Ile Asn

-continued

595

600

<210> SEQ ID NO 49

<211> LENGTH: 545

<212> TYPE: PRT

<213> ORGANISM: Glycine max

<400> SEQUENCE: 49

```

Met  Glu  Thr  Arg  Arg  Ser  Ala  Asn  Tyr  Gln  Pro  Asn  Leu  Trp  Asn  Phe
 1          5          10          15
Glu  Phe  Leu  Pro  Pro  Ser  Leu  Glu  Asn  Asp  His  Lys  Val  Glu  Lys  Leu
          20          25          30
Glu  Glu  Arg  Ala  Arg  Lys  Val  Glu  Glu  Glu  Val  Arg  Arg  Met  Ile  Asn
          35          40          45
Gly  Ala  Asp  Thr  Glu  Ala  Leu  Arg  Leu  Leu  Glu  Leu  Ile  Asp  Glu  Ile
 50          55          60
Gln  Arg  Leu  Gly  Leu  Thr  Tyr  Lys  Phe  Glu  Lys  Asp  Ile  Phe  Lys  Ala
 65          70          75          80
Leu  Glu  Lys  Thr  Ile  Ser  Leu  Asp  Glu  Asn  Glu  Lys  His  Ile  Ser  Gly
          85          90          95
Leu  His  Ala  Thr  Ala  Leu  Ser  Phe  Arg  Leu  Leu  Arg  Gln  His  Gly  Phe
          100          105          110
Glu  Val  Ser  Gln  Asp  Val  Phe  Lys  Arg  Phe  Lys  Asp  Lys  Glu  Gly  Gly
          115          120          125
Phe  Ile  Asn  Glu  Leu  Lys  Gly  Asp  Met  Gln  Gly  Leu  Leu  Ser  Leu  Tyr
          130          135          140
Glu  Ala  Ser  Tyr  Leu  Gly  Phe  Glu  Gly  Glu  Thr  Leu  Leu  Asp  Glu  Ala
          145          150          155          160
Arg  Ala  Tyr  Ser  Ile  Thr  His  Leu  Lys  Asn  Asn  Leu  Lys  Val  Gly  Val
          165          170          175
Asn  Thr  Glu  Val  Lys  Glu  Gln  Val  Ser  His  Ala  Leu  Glu  Leu  Pro  Tyr
          180          185          190
His  Arg  Gly  Leu  Asn  Arg  Leu  Glu  Ala  Arg  Trp  Phe  Leu  Glu  Lys  Tyr
          195          200          205
Glu  Pro  Asn  Glu  Ser  His  His  His  Val  Leu  Leu  Glu  Leu  Ala  Lys  Ile
          210          215          220
Asp  Phe  Asn  Leu  Val  Gln  Val  Met  Tyr  Gln  Lys  Glu  Leu  Arg  Glu  Leu
          225          230          235          240
Ser  Arg  Trp  Trp  Ser  Glu  Met  Gly  Leu  Thr  Ser  Lys  Leu  Lys  Phe  Val
          245          250          255
Arg  Asp  Arg  Leu  Met  Glu  Val  Tyr  Phe  Trp  Val  Leu  Gly  Met  Ala  Pro
          260          265          270
Arg  Pro  Gln  Phe  Ser  Glu  Cys  Arg  Lys  Ala  Val  Thr  Lys  Thr  Phe  Ala
          275          280          285
Leu  Ile  Gly  Ile  Ile  Asp  Asp  Val  Tyr  Asp  Val  Tyr  Gly  Thr  Leu  Asp
          290          295          300
Glu  Leu  Gln  Leu  Phe  Thr  Asp  Ala  Ile  Glu  Arg  Trp  Asp  Val  Asn  Ala
          305          310          315          320
Met  Asn  Thr  Leu  Pro  Asp  Tyr  Met  Lys  Leu  Cys  Tyr  Leu  Ala  Val  Tyr
          325          330          335
Asn  Thr  Val  Asn  Asp  Thr  Cys  Tyr  Ser  Thr  Leu  Lys  Ala  Lys  Gly  His
          340          345          350
Asn  Asn  Met  Ser  Tyr  Leu  Thr  Lys  Ser  Trp  Cys  Glu  Leu  Cys  Lys  Ala
          355          360          365

```

-continued

Phe Leu Gln Glu Ala Lys Trp Ser Asn Asn Lys Ile Val Pro Thr Phe
 370 375 380

Ser Lys Tyr Leu Glu Asn Ala Ser Val Ser Ser Ser Gly Met Ala Leu
 385 390 395 400

Leu Thr Ala Ser Tyr Phe Ser Val Cys Gln Gln Gln Asp Ile Ser Asn
 405 410 415

Gln Gln Ala Leu Cys Ser Leu Thr Asn Phe Gln Gly Leu Val Arg Ser
 420 425 430

Ser Ser Asn Ile Phe Arg Leu Cys Asn Asp Leu Ala Thr Ser Ala Ala
 435 440 445

Glu Leu Glu Thr Gly Glu Thr Ala Asn Ser Ile Thr Cys Tyr Met His
 450 455 460

Glu Lys Asp Thr Ser Glu Glu Gln Ala Arg Glu Glu Leu Thr Asn Leu
 465 470 475 480

Ile Asp Ala Glu Trp Lys Lys Met Asn Arg Glu Phe Val Ser Asn Ser
 485 490 495

Thr Leu Pro Lys Ala Phe Lys Glu Ile Ala Ile Asn Met Ala Arg Val
 500 505 510

Ser His Cys Met Tyr Gln Tyr Glu Asp Gly Leu Gly Arg Pro Gly Tyr
 515 520 525

Thr Thr Glu Asn Lys Ile Lys Leu Leu Leu Ile Asp Pro Val Pro Ile
 530 535 540

Asn
 545

<210> SEQ ID NO 50
 <211> LENGTH: 598
 <212> TYPE: PRT
 <213> ORGANISM: Cajanus cajan

<400> SEQUENCE: 50

Met Ala Thr His His Leu Leu Cys Leu Ser Asn Pro Phe Ser Ser Pro
 1 5 10 15

Ser Pro Thr Leu Ser Thr Ala Thr Arg Ser Phe Pro Leu Thr Asn Asn
 20 25 30

Phe Asn His Lys Thr Ser Leu Ala Asn Ser Lys Pro Cys Pro Phe Ile
 35 40 45

Cys Ser Gln Ile Thr His His His His Thr Arg Arg Ser Ala Asn Tyr
 50 55 60

Gln Pro Asn Leu Trp Asn Phe Glu Phe Leu Gln Ser Leu Gln Asn His
 65 70 75 80

His Gln Val Phe Thr Met Phe Arg Arg Lys Leu Glu Lys Glu Val Arg
 85 90 95

Cys Met Met Asn Lys Ala Asp Ala Glu Ala Leu Ser Leu Leu Glu Leu
 100 105 110

Ile Asp Asp Val Gln Arg Leu Gly Leu Thr Tyr Arg Phe Glu Lys Asp
 115 120 125

Ile Ile Lys Val Leu Glu Lys Ile Val Ser Leu Asp Glu Ile Glu Lys
 130 135 140

His Gln Ser Gly Leu His Ala Thr Ala Leu Thr Phe Arg Leu Leu Arg
 145 150 155 160

Gln His Gly Phe His Gln Val Ser Gln Asp Met Phe Lys Arg Phe Lys
 165 170 175

Asp Lys Glu Gly Gly Phe Asn Asp Glu Leu Lys Gly Asp Val Gln Gly
 180 185 190

-continued

<210> SEQ ID NO 51
 <211> LENGTH: 613
 <212> TYPE: PRT
 <213> ORGANISM: Humulus lupulus

 <400> SEQUENCE: 51

Met Gln Cys Met Ala Val His Gln Phe Ala Pro Leu Leu Ser Leu Leu
 1 5 10 15
 Asn Cys Ser Arg Ile Ser Ser Asp Phe Gly Arg Leu Phe Thr Pro Lys
 20 25 30
 Thr Ser Thr Lys Ser Arg Ser Ser Thr Cys His Pro Ile Gln Cys Thr
 35 40 45
 Val Val Asn Asn Thr Asp Arg Arg Ser Ala Asn Tyr Glu Pro Ser Ile
 50 55 60
 Trp Ser Phe Asp Tyr Ile Gln Ser Leu Thr Ser Gln Tyr Lys Gly Lys
 65 70 75 80
 Ser Tyr Ser Ser Arg Leu Asn Glu Leu Lys Lys Glu Val Lys Met Met
 85 90 95
 Glu Asp Gly Thr Lys Glu Cys Leu Ala Gln Leu Asp Leu Ile Asp Thr
 100 105 110
 Leu Gln Arg Leu Gly Ile Ser Tyr His Phe Glu Asp Glu Ile Asn Thr
 115 120 125
 Ile Leu Lys Arg Lys Tyr Ile Asn Ile Gln Asn Asn Ile Asn His Asn
 130 135 140
 Tyr Asn Leu Tyr Ser Thr Ala Leu Gln Phe Arg Leu Leu Arg Gln His
 145 150 155 160
 Gly Tyr Leu Val Thr Gln Glu Val Phe Asn Ala Phe Lys Asp Glu Thr
 165 170 175
 Gly Lys Phe Lys Thr Tyr Leu Ser Asp Asp Ile Met Gly Val Leu Ser
 180 185 190
 Leu Tyr Glu Ala Ser Phe Tyr Ala Met Lys His Glu Asn Val Leu Glu
 195 200 205
 Glu Ala Arg Val Phe Ser Thr Glu Cys Leu Lys Glu Tyr Met Met Lys
 210 215 220
 Met Glu Gln Asn Lys Val Leu Leu Asp His Asp Leu Asp His Asn Asp
 225 230 235 240
 Asn Phe Asn Val Asn His His Val Leu Ile Ile Asn His Ala Leu Glu
 245 250 255
 Leu Pro Leu His Trp Arg Ile Thr Arg Ser Glu Ala Arg Trp Phe Ile
 260 265 270
 Asp Val Tyr Glu Lys Lys Gln Asp Met Asp Ser Thr Leu Leu Glu Phe
 275 280 285
 Ala Lys Leu Asp Phe Asn Met Val Gln Ser Thr His Gln Glu Asp Leu
 290 295 300
 Lys His Leu Ser Arg Trp Trp Arg His Ser Lys Leu Gly Glu Lys Leu
 305 310 315 320
 Asn Phe Ala Arg Asp Arg Leu Met Glu Ala Phe Leu Trp Glu Val Gly
 325 330 335
 Leu Lys Phe Glu Pro Glu Phe Ser Tyr Phe Lys Arg Ile Ser Ala Arg
 340 345 350
 Leu Phe Val Leu Ile Thr Ile Ile Asp Asp Ile Tyr Asp Val Tyr Gly
 355 360 365
 Thr Leu Glu Glu Leu Glu Leu Phe Thr Lys Ala Val Glu Arg Trp Asp
 370 375 380

-continued

Val Asn Ala Ile Asn Glu Leu Pro Glu Tyr Met Lys Met Pro Phe Leu
 385 390 395 400
 Val Leu His Asn Thr Ile Asn Glu Met Ala Phe Asp Val Leu Gly Asp
 405 410 415
 Gln Asn Phe Leu Asn Ile Glu Tyr Leu Lys Lys Ser Leu Val Asp Leu
 420 425 430
 Cys Lys Cys Tyr Leu Gln Glu Ala Lys Trp Tyr Tyr Ser Gly Tyr Gln
 435 440 445
 Pro Thr Leu Gln Glu Tyr Ile Glu Met Ala Trp Leu Ser Ile Gly Gly
 450 455 460
 Pro Val Ile Leu Val His Ala Tyr Phe Cys Phe Thr Asn Pro Ile Thr
 465 470 475 480
 Lys Glu Ser Met Lys Phe Phe Thr Glu Gly Tyr Pro Asn Ile Ile Gln
 485 490 495
 Gln Ser Cys Leu Ile Val Arg Leu Ala Asp Asp Phe Gly Thr Phe Ser
 500 505 510
 Asp Glu Leu Asn Arg Gly Asp Val Pro Lys Ser Ile Gln Cys Tyr Met
 515 520 525
 Tyr Asp Thr Gly Ala Ser Glu Asp Glu Ala Arg Glu His Ile Lys Phe
 530 535 540
 Leu Ile Cys Glu Thr Trp Lys Asp Met Asn Lys Asn Asp Glu Asp Asn
 545 550 555 560
 Ser Cys Phe Ser Glu Thr Phe Val Glu Val Cys Lys Asn Leu Ala Arg
 565 570 575
 Thr Ala Leu Phe Met Tyr Gln Tyr Gly Asp Gly His Ala Ser Gln Asn
 580 585 590
 Cys Leu Ser Lys Glu Arg Ile Phe Ala Leu Ile Ile Asn Pro Ile Asn
 595 600 605
 Phe His Glu Arg Lys
 610

<210> SEQ ID NO 52
 <211> LENGTH: 56
 <212> TYPE: PRT
 <213> ORGANISM: Quercus petraea

<400> SEQUENCE: 52

Arg Asp Arg Leu Met Glu Cys Phe Phe Phe Phe Ser Phe Ile Thr Val
 1 5 10 15
 Ile Asp Asp Ile Tyr Asp Phe Ser Val Ser Phe Arg Leu Thr Asn Asp
 20 25 30
 Leu Gly Thr Ser Thr Ala Glu Leu Glu Arg Gly Glu Thr Ala Asn Ser
 35 40 45
 Ile Tyr Gln His Gly Asp Gly His
 50 55

<210> SEQ ID NO 53
 <211> LENGTH: 56
 <212> TYPE: PRT
 <213> ORGANISM: Populus alba

<400> SEQUENCE: 53

Arg Asp Arg Leu Ile Glu Ser Phe Tyr Trp Phe Ser Phe Val Thr Ile
 1 5 10 15
 Ile Asp Asp Ile Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
 20 25 30

-continued

Leu Ala Ser Ala Ser Ala Glu Ile Ala Arg Gly Glu Thr Ala Asn Ser
 35 40 45

Val Tyr His Asn Gly Asp Ala His
 50 55

<210> SEQ ID NO 54
 <211> LENGTH: 56
 <212> TYPE: PRT
 <213> ORGANISM: Populus canescens

<400> SEQUENCE: 54

Lys Asp Arg Leu Ile Glu Ser Phe Tyr Trp Phe Ser Phe Val Thr Ile
 1 5 10 15

Ile Asp Asp Ile Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
 20 25 30

Leu Ala Ser Ala Ser Ala Glu Ile Ala Arg Gly Glu Thr Ala Asn Ser
 35 40 45

Val Tyr His Asn Gly Asp Ala His
 50 55

<210> SEQ ID NO 55
 <211> LENGTH: 56
 <212> TYPE: PRT
 <213> ORGANISM: Salix sp.

<400> SEQUENCE: 55

Arg Asp Arg Leu Ile Glu Ser Phe Tyr Trp Phe Ser Phe Val Thr Ile
 1 5 10 15

Ile Asp Asp Ile Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
 20 25 30

Leu Ala Ser Ala Ser Ala Glu Ile Ala Arg Gly Glu Thr Ala Asn Ser
 35 40 45

Val Tyr His Asn Gly Asp Ala His
 50 55

<210> SEQ ID NO 56
 <211> LENGTH: 56
 <212> TYPE: PRT
 <213> ORGANISM: Melaleuca alternifolia

<400> SEQUENCE: 56

Arg Asp Arg Leu Met Glu Cys Phe Phe Trp Phe Ser Leu Ile Leu Val
 1 5 10 15

Leu Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Thr Asn Asp
 20 25 30

Leu Ala Thr Ser Ser Ala Glu Leu Gly Arg Gly Glu Thr Thr Asn Ser
 35 40 45

Ile Tyr Gln Asp Gly Asp Ala Ile
 50 55

<210> SEQ ID NO 57
 <211> LENGTH: 56
 <212> TYPE: PRT
 <213> ORGANISM: Eucalyptus globulus

<400> SEQUENCE: 57

Arg Asp Arg Leu Ile Glu Cys Phe Phe Trp Phe Ala Leu Ile Leu Val
 1 5 10 15

Leu Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Thr Asn Asp

-continued

```

      20          25          30
Ile Ala Ser Ser Ser Ala Glu Leu Glu Arg Gly Glu Thr Thr Asn Ser
      35          40          45

```

```

Ile Tyr Gln Asp Gly Asp Ala Ile
      50          55

```

```

<210> SEQ ID NO 58
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Wisteria sp.

```

```

<400> SEQUENCE: 58

```

```

Arg Asp Arg Leu Met Glu Val Tyr Phe Trp Phe Gly Leu Val Thr Ile
1          5          10          15

```

```

Ile Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
      20          25          30

```

```

Leu Ala Thr Ser Ala Ala Glu Leu Glu Arg Gly Glu Thr Thr Asn Ser
      35          40          45

```

```

Ile Tyr Gln His Gly Asp Gly Leu
      50          55

```

```

<210> SEQ ID NO 59
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Robinia pseudoacacia

```

```

<400> SEQUENCE: 59

```

```

Arg Asp Arg Leu Val Glu Val Tyr Phe Trp Phe Gly Leu Val Thr Ile
1          5          10          15

```

```

Ile Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
      20          25          30

```

```

Leu Ala Thr Ser Ala Ala Glu Leu Glu Arg Gly Glu Thr Thr Asn Ser
      35          40          45

```

```

Ile Tyr Gln Tyr Gly Asp Gly Leu
      50          55

```

```

<210> SEQ ID NO 60
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Arachis hypogaea

```

```

<400> SEQUENCE: 60

```

```

Arg Asp Arg Leu Met Glu Val Tyr Phe Trp Phe Gly Leu Val Thr Ile
1          5          10          15

```

```

Ile Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
      20          25          30

```

```

Leu Ala Thr Ser Ala Ala Glu Leu Glu Arg Gly Glu Thr Thr Asn Ser
      35          40          45

```

```

Ile Tyr Gln Tyr Gly Asp Gly Leu
      50          55

```

```

<210> SEQ ID NO 61
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Glycine max

```

```

<400> SEQUENCE: 61

```

```

Arg Asp Arg Leu Met Glu Val Tyr Phe Trp Phe Gly Leu Val Thr Ile
1          5          10          15

```

-continued

```

Ile Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
      20                               25                               30
Leu Thr Thr Ser Ala Ala Glu Leu Glu Arg Gly Glu Thr Thr Asn Ser
      35                               40                               45
Ile Tyr Gln Tyr Gly Asp Gly Leu
      50                               55

```

```

<210> SEQ ID NO 62
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Pueraria montana var. lobata

```

```

<400> SEQUENCE: 62

```

```

Arg Asp Arg Leu Met Glu Val Tyr Phe Trp Phe Gly Leu Val Thr Ile
 1      5      10      15
Ile Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
      20                               25                               30
Leu Ala Thr Ser Ala Ala Glu Leu Glu Arg Gly Glu Thr Thr Asn Ser
      35                               40                               45
Ile Tyr Gln Tyr Gly Asp Gly Leu
      50                               55

```

```

<210> SEQ ID NO 63
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Mucuna pruriens

```

```

<400> SEQUENCE: 63

```

```

Arg Asp Arg Leu Met Glu Val Tyr Phe Trp Phe Gly Leu Val Thr Ile
 1      5      10      15
Ile Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
      20                               25                               30
Leu Ala Thr Ser Ala Ala Glu Leu Glu Arg Gly Glu Thr Thr Asn Ser
      35                               40                               45
Ile Tyr Gln Tyr Gly Asp Gly Leu
      50                               55

```

```

<210> SEQ ID NO 64
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Glycine max

```

```

<400> SEQUENCE: 64

```

```

Arg Asp Arg Leu Met Glu Val Tyr Phe Trp Phe Ala Leu Ile Gly Ile
 1      5      10      15
Ile Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
      20                               25                               30
Leu Ala Thr Ser Ala Ala Glu Leu Glu Thr Gly Glu Thr Ala Asn Ser
      35                               40                               45
Ile Tyr Gln Tyr Glu Asp Gly Leu
      50                               55

```

```

<210> SEQ ID NO 65
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Cajanus cajan

```

```

<400> SEQUENCE: 65

```

```

Arg Asp Arg Leu Met Glu Val Tyr Phe Trp Phe Gly Leu Val Thr Ile
 1      5      10      15

```


-continued

```

1           5           10           15
Ile Asp Asp Ile Tyr Asp Phe Ser Val Ser Phe Arg Leu Cys Asn Asp
      20           25           30
Leu Gly Thr Ser Thr Ala Glu Leu Gln Arg Gly Glu Val Ala Asn Ser
      35           40           45
Ile Tyr Gln Thr Gly Asp Gly His
      50           55

```

```

<210> SEQ ID NO 70
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Petraea sp.

```

```

<400> SEQUENCE: 70

```

```

Arg Asp Arg Leu Met Glu Cys Phe Phe Phe Phe Ser Phe Ile Thr Val
1           5           10           15
Ile Asp Asp Ile Tyr Asp Phe Ser Val Ser Phe Arg Leu Thr Asn Asp
      20           25           30
Leu Gly Thr Ser Thr Ala Glu Leu Glu Arg Gly Glu Thr Ala Asn Ser
      35           40           45
Ile Tyr Gln His Gly Asp Gly His
      50           55

```

```

<210> SEQ ID NO 71
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Fragaria vesca

```

```

<400> SEQUENCE: 71

```

```

Lys Asp Arg Leu Met Glu Cys Phe Phe Trp Ser Ala Leu Ile Ser Thr
1           5           10           15
Val Asp Asp Val Tyr Asp Phe Ser Ala Ser Phe Arg Leu Thr Asn Asp
      20           25           30
Leu Ala Thr Ser Glu Ala Glu Leu Glu Arg Gly Glu Thr Ala Asn Ser
      35           40           45
Ile Tyr Gln Tyr Gly Asp Gly Ile
      50           55

```

```

<210> SEQ ID NO 72
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Morus notabilis

```

```

<400> SEQUENCE: 72

```

```

Arg Asp Arg Leu Met Glu Ser Phe Phe Trp Val Ala Phe Ile Thr Val
1           5           10           15
Ile Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Val Asn Asp
      20           25           30
Leu Ala Thr Ser Thr Ala Glu Leu Glu Arg Gly Glu Thr Asn Asn Ser
      35           40           45
Ile Tyr Gln His Gly Asp Gly His
      50           55

```

```

<210> SEQ ID NO 73
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Elaeocarpus sp.

```

```

<400> SEQUENCE: 73

```

-continued

```

Arg Asp Arg Leu Met Glu Ser Phe Phe Trp Phe Ala Leu Ile Thr Thr
1          5          10          15
Ile Asp Asp Val Tyr Asp Phe Ser Val Ser Phe Arg Leu Cys Asn Asp
          20          25          30
Leu Gly Thr Ser Ser Ala Glu Leu Glu Arg Gly Glu Leu Ala Asn Ser
          35          40          45
Ile Tyr Gln Tyr Gly Asp Ala His
          50          55

```

```

<210> SEQ ID NO 74
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Elaeocarpus photiniifolius

```

```

<400> SEQUENCE: 74

```

```

Arg Asp Arg Leu Met Glu Ser Phe Phe Trp Phe Ala Leu Ile Thr Thr
1          5          10          15
Ile Asp Asp Val Tyr Asp Phe Ser Val Ser Phe Arg Leu Cys Asn Asp
          20          25          30
Leu Gly Thr Ser Ser Ala Glu Leu Glu Arg Gly Glu Leu Ala Asn Ser
          35          40          45
Ile Tyr Gln Tyr Gly Asp Ala His
          50          55

```

```

<210> SEQ ID NO 75
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Populus trichocarpa

```

```

<400> SEQUENCE: 75

```

```

Arg Asp Arg Leu Met Glu Cys Phe Phe Trp Thr Ser Phe Ile Thr Thr
1          5          10          15
Ile Asp Asp Val Tyr Asp Phe Ser Val Ser Phe Arg Leu Ser Asn Asp
          20          25          30
Leu Ala Thr Ser Ser Ala Glu Ile Ala Arg Gly Glu Thr Ala Asn Ser
          35          40          45
Ile Tyr Gln His Gly Asp Gly His
          50          55

```

```

<210> SEQ ID NO 76
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Vitis vinifera

```

```

<400> SEQUENCE: 76

```

```

Arg Asp Arg Leu Met Glu Cys Phe Phe Trp Thr Ser Phe Ile Thr Thr
1          5          10          15
Ile Asp Asp Val Tyr Asp Phe Ser Val Ser Phe Arg Leu Cys Asn Asp
          20          25          30
Leu Ala Thr Ser Lys Ala Glu Leu Glu Arg Gly Glu Ser Ala Asn Ser
          35          40          45
Ile Tyr Gln Tyr Gly Asp Ser His
          50          55

```

```

<210> SEQ ID NO 77
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Populus alba

```

```

<400> SEQUENCE: 77

```

-continued

```

Arg Asp Arg Leu Ile Glu Ser Phe Tyr Trp Phe Ser Phe Val Thr Ile
1           5           10           15
Ile Asp Asp Ile Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
20           25           30
Leu Ala Ser Ala Ser Ala Glu Ile Ala Arg Gly Glu Thr Ala Asn Ser
35           40           45
Val Tyr His Asn Gly Asp Ala His
50           55

```

```

<210> SEQ ID NO 78
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Populus canescens

```

```

<400> SEQUENCE: 78

```

```

Lys Asp Arg Leu Ile Glu Ser Phe Tyr Trp Phe Ser Phe Val Thr Ile
1           5           10           15
Ile Asp Asp Ile Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
20           25           30
Leu Ala Ser Ala Ser Ala Glu Ile Ala Arg Gly Glu Thr Ala Asn Ser
35           40           45
Val Tyr His Asn Gly Asp Ala His
50           55

```

```

<210> SEQ ID NO 79
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Salix sp.

```

```

<400> SEQUENCE: 79

```

```

Arg Asp Arg Leu Ile Glu Ser Phe Tyr Trp Phe Ser Phe Val Thr Ile
1           5           10           15
Ile Asp Asp Ile Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
20           25           30
Leu Ala Ser Ala Ser Ala Glu Ile Ala Arg Gly Glu Thr Ala Asn Ser
35           40           45
Val Tyr His Asn Gly Asp Ala His
50           55

```

```

<210> SEQ ID NO 80
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Malternifolia sp.

```

```

<400> SEQUENCE: 80

```

```

Arg Asp Arg Leu Met Glu Cys Phe Phe Trp Phe Ser Leu Ile Leu Val
1           5           10           15
Leu Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Thr Asn Asp
20           25           30
Leu Ala Thr Ser Ser Ala Glu Leu Gly Arg Gly Glu Thr Thr Asn Ser
35           40           45
Ile Tyr Gln Asp Gly Asp Ala Ile
50           55

```

```

<210> SEQ ID NO 81
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Eglobulus sp.

```

-continued

<400> SEQUENCE: 81

```

Arg Asp Arg Leu Ile Glu Cys Phe Phe Trp Phe Ala Leu Ile Leu Val
1           5           10           15
Leu Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Thr Asn Asp
20           25           30
Ile Ala Ser Ser Ser Ala Glu Leu Glu Arg Gly Glu Thr Thr Asn Ser
35           40           45
Ile Tyr Gln Asp Gly Asp Ala Ile
50           55

```

<210> SEQ ID NO 82

<211> LENGTH: 56

<212> TYPE: PRT

<213> ORGANISM: *Eucalyptus grandis*

<400> SEQUENCE: 82

```

Arg Asp Arg Leu Thr Glu Cys Phe Phe Trp Thr Ser Leu Ile Thr Ile
1           5           10           15
Met Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Thr Asn Asp
20           25           30
Leu Val Thr Leu Ser Ala Glu Leu Glu Arg Gly Glu Thr Thr Asn Ser
35           40           45
Ile Tyr Gln Asp Gly Asp Ala His
50           55

```

<210> SEQ ID NO 83

<211> LENGTH: 56

<212> TYPE: PRT

<213> ORGANISM: *Wisteria sp.*

<400> SEQUENCE: 83

```

Arg Asp Arg Leu Met Glu Val Tyr Phe Trp Phe Gly Leu Val Thr Ile
1           5           10           15
Ile Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
20           25           30
Leu Ala Thr Ser Ala Ala Glu Leu Glu Arg Gly Glu Thr Thr Asn Ser
35           40           45
Ile Tyr Gln His Gly Asp Gly Leu
50           55

```

<210> SEQ ID NO 84

<211> LENGTH: 56

<212> TYPE: PRT

<213> ORGANISM: *Robinia pseudoacacia*

<400> SEQUENCE: 84

```

Arg Asp Arg Leu Val Glu Val Tyr Phe Trp Phe Gly Leu Val Thr Ile
1           5           10           15
Ile Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
20           25           30
Leu Ala Thr Ser Ala Ala Glu Leu Glu Arg Gly Glu Thr Thr Asn Ser
35           40           45
Ile Tyr Gln Tyr Gly Asp Gly Leu
50           55

```

<210> SEQ ID NO 85

<211> LENGTH: 56

<212> TYPE: PRT

<213> ORGANISM: *Arachis hypogaea*

-continued

<400> SEQUENCE: 85

```

Arg Asp Arg Leu Met Glu Val Tyr Phe Trp Phe Gly Leu Val Thr Ile
1           5           10           15
Ile Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
           20           25           30
Leu Ala Thr Ser Ala Ala Glu Leu Glu Arg Gly Glu Thr Thr Asn Ser
           35           40           45
Ile Tyr Gln Tyr Gly Asp Gly Leu
           50           55

```

<210> SEQ ID NO 86

<211> LENGTH: 56

<212> TYPE: PRT

<213> ORGANISM: Glycine max

<400> SEQUENCE: 86

```

Arg Asp Arg Leu Met Glu Val Tyr Phe Trp Phe Gly Leu Val Thr Ile
1           5           10           15
Ile Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
           20           25           30
Leu Thr Thr Ser Ala Ala Glu Leu Glu Arg Gly Glu Thr Thr Asn Ser
           35           40           45
Ile Tyr Gln Tyr Gly Asp Gly Leu
           50           55

```

<210> SEQ ID NO 87

<211> LENGTH: 56

<212> TYPE: PRT

<213> ORGANISM: Pueraria montana var. lobata

<400> SEQUENCE: 87

```

Arg Asp Arg Leu Met Glu Val Tyr Phe Trp Phe Gly Leu Val Thr Ile
1           5           10           15
Ile Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
           20           25           30
Leu Ala Thr Ser Ala Ala Glu Leu Glu Arg Gly Glu Thr Thr Asn Ser
           35           40           45
Ile Tyr Gln Tyr Gly Asp Gly Leu
           50           55

```

<210> SEQ ID NO 88

<211> LENGTH: 56

<212> TYPE: PRT

<213> ORGANISM: Mucuna pruriens

<400> SEQUENCE: 88

```

Arg Asp Arg Leu Met Glu Val Tyr Phe Trp Phe Gly Leu Val Thr Ile
1           5           10           15
Ile Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
           20           25           30
Leu Ala Thr Ser Ala Ala Glu Leu Glu Arg Gly Glu Thr Thr Asn Ser
           35           40           45
Ile Tyr Gln Tyr Gly Asp Gly Leu
           50           55

```

<210> SEQ ID NO 89

<211> LENGTH: 56

<212> TYPE: PRT

-continued

<213> ORGANISM: *Glycine max*

<400> SEQUENCE: 89

Arg Asp Arg Leu Met Glu Val Tyr Phe Trp Phe Ala Leu Ile Gly Ile
 1 5 10 15
 Ile Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
 20 25 30
 Leu Ala Thr Ser Ala Ala Glu Leu Glu Thr Gly Glu Thr Ala Asn Ser
 35 40 45
 Ile Tyr Gln Tyr Glu Asp Gly Leu
 50 55

<210> SEQ ID NO 90

<211> LENGTH: 56

<212> TYPE: PRT

<213> ORGANISM: *Cajanus cajan*

<400> SEQUENCE: 90

Arg Asp Arg Leu Met Glu Val Tyr Phe Trp Phe Gly Leu Val Thr Ile
 1 5 10 15
 Ile Asp Asp Val Tyr Asp Phe Ser Ser Ser Phe Arg Leu Tyr Asn Asp
 20 25 30
 Leu Ala Thr Ser Ala Ala Glu Leu Glu Arg Asp Glu Thr Thr Asn Ser
 35 40 45
 Met Tyr Gln His Gly Asp Gly Leu
 50 55

<210> SEQ ID NO 91

<211> LENGTH: 56

<212> TYPE: PRT

<213> ORGANISM: *Mangifera indica*

<400> SEQUENCE: 91

Arg Asp Arg Leu Met Glu Cys Tyr Phe Trp Phe Ala Phe Val Thr Thr
 1 5 10 15
 Ile Asp Asp Ile Tyr Asp Phe Ser Ser Ser Phe Arg Leu Cys Asn Asp
 20 25 30
 Leu Ser Thr Ser Lys Asp Glu Leu Glu Arg Gly Glu Thr Ala Ser Ser
 35 40 45
 Ile Tyr Gln His Gly Asp Gly His
 50 55

<210> SEQ ID NO 92

<211> LENGTH: 56

<212> TYPE: PRT

<213> ORGANISM: *Ipomoea batatas*

<400> SEQUENCE: 92

Arg Asp Arg Leu Met Glu Ser Phe Phe Trp Phe Lys Leu Val Thr Val
 1 5 10 15
 Leu Asp Asp Val Tyr Asp Phe Ser Val Ser Phe Arg Leu Ala Asn Asp
 20 25 30
 Leu Ser Ser Ser Lys Ala Glu Ile Glu Arg Gly Glu Thr Ala Asn Ser
 35 40 45
 Ile Tyr Gln Tyr Gly Asp Ala His
 50 55

<210> SEQ ID NO 93

<211> LENGTH: 56

-continued

```

<212> TYPE: PRT
<213> ORGANISM: Sesamum indicum

<400> SEQUENCE: 93
Arg Asp Arg Leu Met Glu Ser Phe Phe Trp Val Asn Leu Ile Thr Val
1          5          10          15
Leu Asp Asp Ile Tyr Asp Phe Ser Ser Ser Phe Arg Leu Ala Asn Asp
          20          25          30
Leu Ser Ser Ser Lys Asp Asp Val Gly Arg Gly Glu Thr Ala Lys Ala
          35          40          45
Val Tyr Gln His Gly Asp Ala His
          50          55

<210> SEQ ID NO 94
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Humulus lupulus

<400> SEQUENCE: 94
Arg Asp Arg Leu Met Glu Ala Phe Leu Trp Phe Val Leu Ile Thr Ile
1          5          10          15
Ile Asp Asp Ile Tyr Asp Tyr Ser Ile Gly Val Arg Leu Ala Asp Asp
          20          25          30
Phe Gly Thr Phe Ser Asp Glu Leu Asn Arg Gly Asp Val Pro Lys Ser
          35          40          45
Ile Tyr Gln Tyr Gly Asp Gly His
          50          55

<210> SEQ ID NO 95
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Matricaria recutita

<400> SEQUENCE: 95
Arg Asp Arg Leu Met Glu Cys Phe Phe Trp Ala Thr Leu Ile Thr Thr
1          5          10          15
Ile Asp Asp Ile Tyr Asp Phe Ser Val Ser Phe Arg Leu Tyr Asn Asp
          20          25          30
Leu Ala Ala Leu Ala Asp Glu Ile Asp Lys Asp Lys Ser Pro Asn Ala
          35          40          45
Ile Tyr Gln Tyr Gly Asp Gly Ile
          50          55

<210> SEQ ID NO 96
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Dahlia pinnata

<400> SEQUENCE: 96
Arg Asp Arg Leu Leu Glu Cys Phe Phe Trp Ser Thr Phe Ile Thr Ile
1          5          10          15
Leu Asp Asp Ile Tyr Asp Phe Ser Val Ser Phe Arg Leu Tyr Asn Asp
          20          25          30
Leu Ala Thr Ser Ser Ser Glu Ile Gln Arg Gly Lys Asn Val Asn Ala
          35          40          45
Val Tyr Gln Tyr Gly Asp Gly His
          50          55

```

What is claimed is:

1. A recombinant cell comprising an isoprene synthase enzyme having 95% or more sequence identity to SEQ ID NO: 1, wherein the recombinant cell is a cyanobacteria, yeast, or filamentous fungi. 5
2. The recombinant cell of claim 1, wherein the isoprene synthase enzyme has 98% or more sequence identity to SEQ ID NO: 1.
3. The recombinant cell of claim 1, wherein the isoprene synthase enzyme has 99% or more sequence identity to SEQ 10 ID NO: 1.

* * * * *